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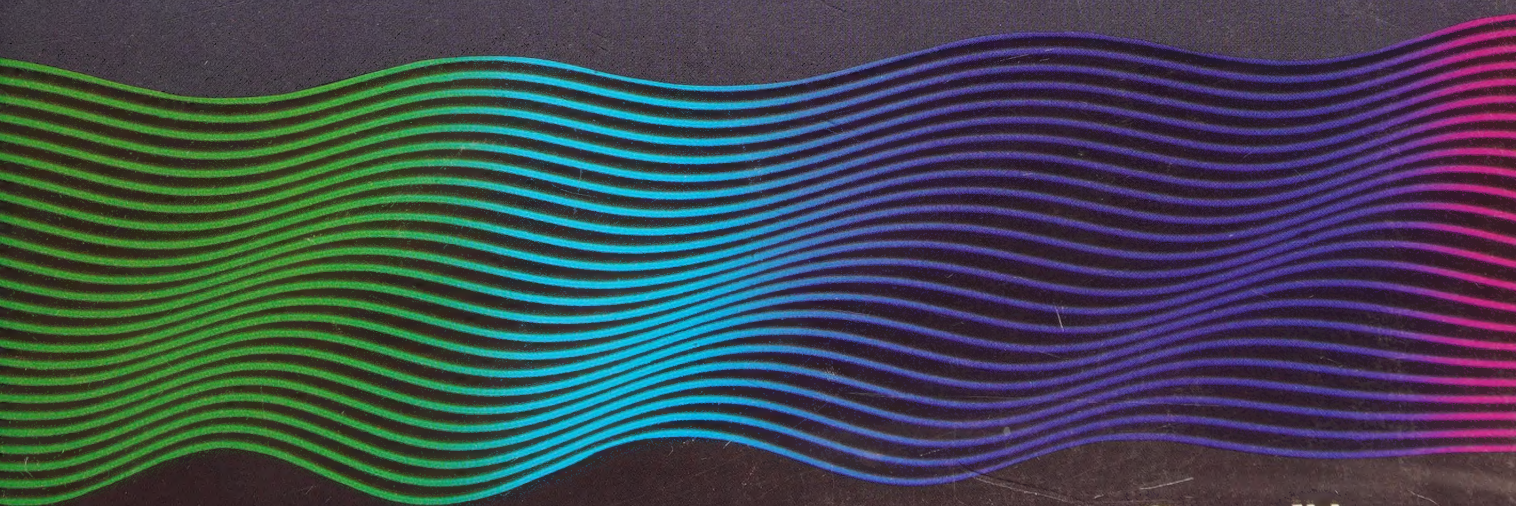

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An Acoustic Study of Shrimp (*Pandalus montagui*) Distribution near Resolution Island (Eastern Hudson Strait)

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Crawford, R. E., C. Hudon, and D. G. Parsons. 1992. An acoustic study of shrimp (*Pandalus montagui*) distribution near Resolution Island (eastern Hudson Strait) Can. J. Fish. Aquat. Sci. 49: 842–856.

Echo integration, a multistage plankton sampler (BIONESS), and a bottom trawl were used to examine the horizontal and vertical distribution of shrimp (*Pandalus montagui*) near Resolution Island in eastern Hudson Strait. Shrimp were concentrated in two locations within the study area and they maintained this pattern of horizontal distribution for at least 7 d. Acoustic observations revealed a scale of horizontal patchiness that was obscured by the "homogenization effect" of sampling by bottom trawl and plankton net. The shrimp underwent a nocturnal vertical migration with other zooplankton to >200 m from the bottom and a subsequent downward migration during early daylight hours. This diel migration resulted in reduced availability of shrimp to the bottom trawl at night. Timing of the migrations varied, possibly as a result of interaction with oceanographic processes.

On a étudié les distributions horizontale et verticale de la crevette *Pandalus montagui* au moyen d'un échointégrateur, d'un système de filets à plancton multiples (BIONESS) et d'un chalut de fond près de l'île Resolution, située dans la partie est du détroit d'Hudson. Les crevettes étaient concentrées en deux endroits de l'aire d'étude et ont gardé cette distribution horizontale durant au moins 7 jours. Les données acoustiques ont montré l'existence d'une répartition horizontale non uniforme à petite échelle, masquée par l'effet homogénéisant de l'échantillonnage au chalut de fond et au filet à plancton. Les crevettes migrent verticalement la nuit, avec d'autres organismes zooplanctoniques, jusqu'à une distance de plus de 200 m du fond, puis redescendent dès les premières heures du jour. Le chalut de fond récoltait moins de crevettes durant la nuit en raison de cette migration nycthémérale. Les migrations ascendantes et descendantes des crevettes n'avaient pas toujours lieu aux mêmes heures; les processus océanographiques en sont peut-être responsables.

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The diel cyclic fluctuations of pandalid shrimp trawl catch rates are well known to fishermen and have been attributed to the vertical movements of shrimp (Carlsson et al. 1978; Fréchette et al. 1984; Shumway et al. 1985). Catch rates typically reach their minimum values at night when the shrimp move off the bottom. Catch patterns have suggested that, on certain days, the pelagic phase of this migration can be extensive, with proportionately more time spent in the water column than on the bottom. In addition, the cyclic pattern of repetitive catches at one location may not be predictable over an extended period (D. G. Parsons, Northwest Atlantic Fisheries Centre, P.O. Box 5667, St. John's, Nfld. A1C 5X1, unpubl. data). Diel migration has been described for the northern shrimp (*Pandalus borealis*) (reviewed by Shumway et al. 1985), primarily from catches of baited traps suspended in the water column and from net samples. Light intensity appears to be the predominant influence on the diel migration cycle, and seasonal variation in the magnitude of diurnal changes in catch rates has been observed (Smidt 1978). Variation in bottom trawl catches of northern shrimp and striped pink shrimp (*P. montagui*) during stock assessment surveys in Hudson Strait and near Labrador (D. G. Parsons, unpubl. data) suggested that diel migration may be an important component of shrimp ecology in this region. The impact of this behaviour on stock assessment and the commercial fisheries was unknown.

We examined the hypothesis that short-term variations in the pattern of *P. montagui* distribution were affecting stock assessments made with bottom trawls. It was postulated that if the shrimp underwent diel migration, their availability to bottom sampling equipment would be affected. Also, in addition to executing studies of shrimp ecology and biology, we examined the scale of patchiness in their horizontal distribution, from the perspective of the potential influence of the behaviour of shrimp aggregations on swept area biomass estimates derived from bottom trawl surveys. We used fisheries acoustics methods, a multiple-net plankton sampler, and a bottom trawl. The implications for stock assessment, as well as examinations of shrimp community feeding ecology and the effects of physical forcing

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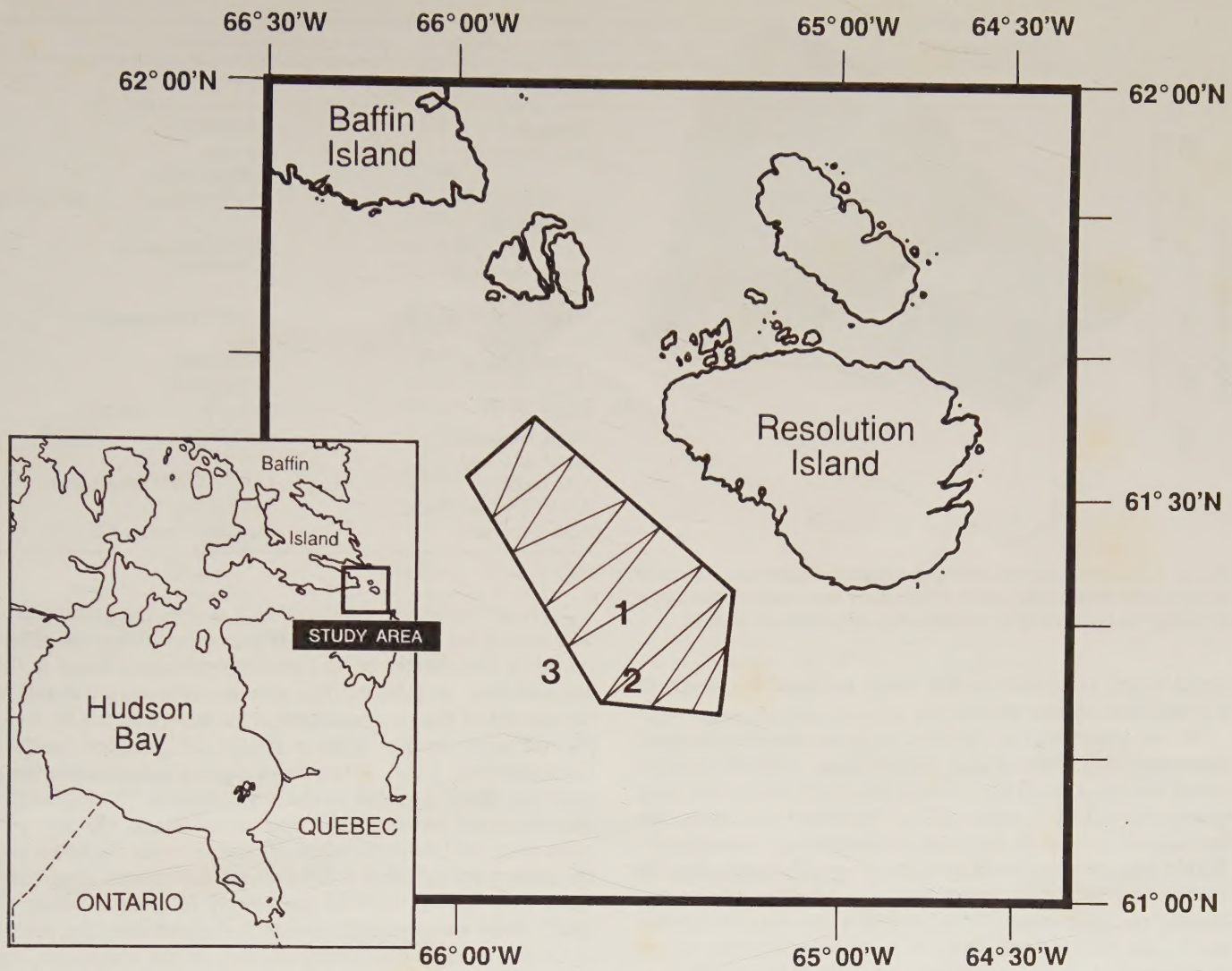

FIG. 1. Study areas for *P. montagui* in eastern Hudson Strait, August 22 to September 2, 1988. Zigzag lines represent the acoustic survey track. Numbers indicate the location of BIONESS series sampling sites.

on their distribution, will be reported elsewhere. This paper presents the first detailed, acoustically derived description of shrimp diel migration.

Materials and Methods

Sampling was carried out August 22 to September 2, 1988, in eastern Hudson Strait (Fig. 1) where high concentrations of striped pink shrimp have been exploited commercially since 1979 (Parsons et al. 1987). The study was done in an area characterized by high tidal amplitudes (Canadian Hydrographic Service 1988) and swift currents (Canadian Hydrographic Service 1983). The location was in a transition zone between the stratified waters of central Hudson Strait and the highly mixed waters of Davis Strait (Drinkwater and Jones 1987). The primary survey area was an 802.6-km^2 pentagon (Fig. 2) on a slope southwest of Resolution Island where research and commercial shrimp catches of previous years have been concentrated (Hudon 1990). Species diversity in the area is low and characteristically Arctic (Hudon 1990).

Echo Integration

The acoustic system used a 120-kHz echosounder (BioSonics Inc.). It included a single-beam transducer in a vee-fin towed body and an uncalibrated transmission cable (about 100 m long). The vee-fin was towed between 26 and 87 m beneath the surface. System operating parameters (Table 1) were estimated from a laboratory calibration conducted immediately prior to the cruise, albeit with a different, shorter transmission cable (73 m) of similar impedance (50 Ω). Measurements made after the study indicated that the two cables had similar transmission line characteristics (G. Lobb, Maurice-Lamontagne Institute, Mont-Joli, Que., pers. comm.). The accuracy of the acoustic system (and subsequent study results) was estimated to be near that recommended by Pope (1982) for exploratory surveys (±3 dB).

We derived a value for the acoustic target strength (TS) of the striped pink shrimp from an evaluation of the literature. Most measurements of crustacean acoustic backscatter have been done on euphausiids and nonpandalid shrimps. This work has produced a wide range of values for planktonic macrocrustaceans (Table 2). We made the a priori assumption that there was similarity between the acoustic characteristics of euphausiids and pandalids because their shapes were similar. We elected to use the euphausiid TS relationshp (Table 2) adopted by the first international BIOMASS experiment (FIBEX) acoustic working group (Anonymous 1986). The

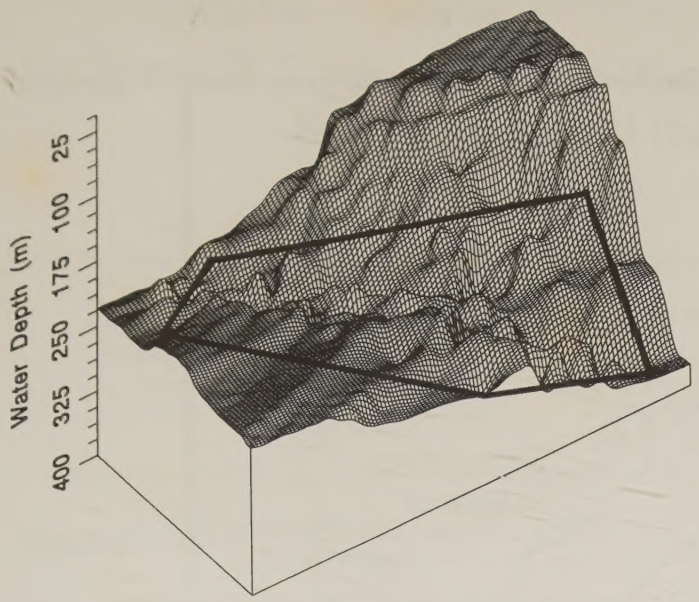

FIG. 2. Landscape derived from water depth measurements obtained during the hydroacoustic survey of the study area (outline indicated), revealing the presence of a trench and coherent bottom features.

length/weight component of this model predicted the weight of a striped pink shrimp within 9%.

We also presumed that pandalid shrimp are directional sound scatterers, in the manner of euphausiids and sergestid shrimps whose frontal aspect TS was about 20–30 dB lower than peak dorsal aspect TS (Greenlaw 1977). Stanton (1987) noted that the acoustic length of a shrimp is less than its total length. Within our samples (mean total length = 10.2 cm), the thin rostrum was 24% of the shrimp's total length. We assumed that the rostrum contributed little to the backscattering cross section at 120 kHz and subtracted it from the total length (mean acoustic length = 7.76 cm). The resulting predicted peak TS of an individual shrimp was −58.0 dB. This value was preferred to the most commonly cited "shrimp" TS of −50 dB (Sofoulis et al. 1979) because the latter was obtained from two large specimens of penaeid prawns. Penrose and Kaye (1979) found that crustacean TS values fell about 6 dB lower than that predicted for a similarly sized air-bladdered fish (Love 1977). Our derived TS fell within this expected range and compared favourably with those for other crustaceans (Fig. 3). At an individual mean weight of 5.7 g, and in accordance with all of the above assumptions, we derived a transformed (Williamson and Traynor 1984) mean weight-specific TS of -42.2 dB·kg^{-1} (sigma = 0.6026×10^{-4}·kg^{-1}·m^{-2}).

During echo integration analysis, we observed an apparent decrease in biomass when comparing data collected at night with data collected during the daytime. Everson (1982) noted similar results for euphausiids. He assumed that they had adopted a different swimming behaviour during nocturnal feeding activities which changed their mean swimming aspect in relation to a downward-looking transducer. This change in aspect altered their mean TS when compared with daytime "nonfeeding" values. He accounted for the difference with a 8.54-dB nocturnal reduction in krill TS. We followed this conjecture but applied a conservative 6-dB nocturnal decrease in TS to our data because the decrease we observed was less than that reported by Everson (1982). The derived nocturnal value was -48.2 dB·kg^{-1} (sigma = 0.1514×10^{-4}·kg^{-1}·m^{-2}).

TABLE 1. Echo sounder/echo integration system specifications and calibration data.

Transmitter/ receiver	BioSonics model 101
Frequency	120 kHz
Transducer	Circular, single-beam, 10° (nominal, −3 dB points)
Source level (dB·μPa^{-1} at 1 m)	218.9 (estimated)
System-receiving sensitivity (dBv·μPa^{-1} at 1 m)	−162.5 (estimated)
Pulse length	2 ms
Pulse repetition rate	66.7·min^{-1}
TVG	20 log R (25–250 m)
Water temperature	0.5°C
Speed of sound in water	1445.7 m·s^{-1} (estimated)
Absorption coefficient	34.7 dB·km^{-1}
Echo integrator	BioSonics model 121

Shrimp biomass was estimated with an echo integrator scaled with one of the two TS values. Which value to use was determined by the amount of background reverbatory noise in the acoustic data, as follows. The acoustic data were indexed to the amount of macrozooplankton in the water column by comparison with samples from a BIONESS plankton sampler (Sameoto et al. 1980). When the catches of macrozooplankton were nil, there remained a relatively constant "background" acoustic signal that ranged between about 80 and 150 mg·m^{-2} when converted with TS values. The source was unknown and the noise level followed a nonuniform diel pattern (e.g. transition from low to high did not strictly follow the timing of dusk). Noise was generally constant for a given sampling period and higher at night than during the day. When it was high, the nocturnal TS value was used. In all cases, the amplitude of biological noise was a small percentage of the biomass signals indicative of schools of shrimp, which were common. Noise was subtracted from data prior to biomass transformation.

Target depth was determined by adding the transducer depth to target range (distance from transducer) values. When target range exceeded the 250-m limit of the receiver's time-varied gain (TVG) circuitry, the data were adjusted with postprocessing software. We eliminated electrical and nonreverbatory acoustical noise from the echo integration process according to the method of Nunnallee (1987). Signal "thresholding" was not used.

The fidelity of the echo integration analysis was verified using graphical data visualization (Crawford and Fox 1992). This was also used during the data editing process when checking for echoes of the BIONESS plankton sampler.

Biomass estimates derived acoustically are hereafter referred to as "acoustic biomass" estimates. The acoustic biomass contained within a particular depth range and beneath a defined surface area (grams per square metre) is the "surface density." The biomass within a volume (grams per cubic metre) is the "volume density."

Horizontal Distribution of Biomass

The pentagonal study area was examined acoustically in a survey consisting of 13 transects in a zigzag pattern (Fig. 1).

TABLE 2. Target strengths (TS) for several large planktonic crustaceans, as reported in the literature. Model used for this study in bold type. L = length (mm), W = wet weight (g), F = frequency (hz).

Organism	TS	Source
Euphausiid	$-72.0 + 2.3\,L$	Kalinovsky et al. 1979, cited in Thiel 1984
Euphausiid	−65 dB	Sameoto 1980
Euphausiid	−81.3 dB (daytime, −89.2 dB (nighttime)	Everson 1982
Euphausiid	−65.1 dB	Guzman et al. 1982
Euphausiid	$-72.3 + 2.1\,L$	Protaschuk and Lukashova 1982
Euphausiid	$-59.38 + 6.70 \log W$ $-75.1 + 23.05 \log L$	Sasakura et al. 1982
Euphausiid	**$-95.7 + 19.9 \log L$**	**Anonymous 1986 (FIBEX)**
Euphausiid (33.7 mm)	−89 to −85 dB @ 38 kHz −79 to −76 dB @ 120 kHz	Foote et al. 1990
Freshwater shrimp	−50 dB	Thiel 1984
Sergestid shrimp	−78 dB (dorsal), −90.5 dB (anterior)	Greenlaw 1977
Penaeid shrimp	$-44.4 - (2.52 \times 10^{-3} F)$	Penrose and Kaye 1979
Penaeid shrimp	Peak = $-44.4 - (2.52 \times 10^{-3} F)$, mean = $-51.1 - (1.68 \times 10^{-3} F)$	Sofoulis et al. 1979
Crustacean zooplankton	$-107.7 + 26.6 \log(L)$	Greene et al. 1989

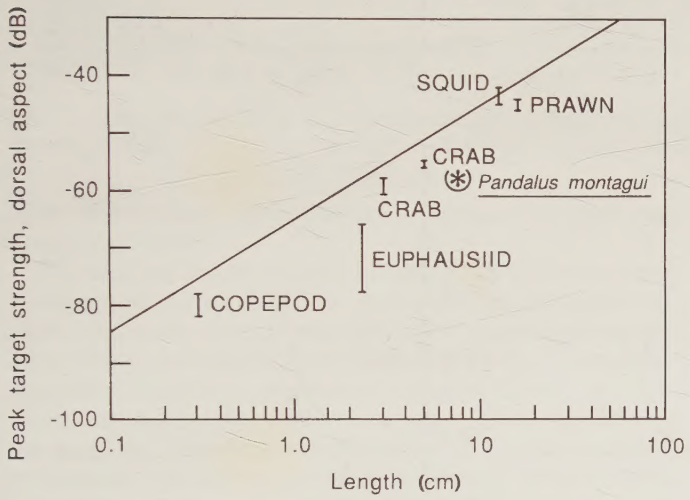

FIG. 3. Association of derived peak TS for pandalid shrimp with those determined for other organisms. The diagonal is the theoretical relationship for fishes with swimbladders (from Love 1977). Figure modified after Penrose and Kaye (1979).

Average vessel speed was about 8.3 km·h^{-1}. Data were collected in segments about 460 m long (3.3 min each). Biomass distribution was mapped with graphical software (SURFER program, Golden Software, Golden, CO) using surface density values derived for all the segments of the transects.

The total acoustic biomass within the study area was estimated from a poststratification analysis as follows. The area was divided into 13 parts, each containing one transect passing diagonally across it. Distribution within each part was assumed to be homogeneous. Echo integration was used to determine surface density within two depth zones of each part: 0–7 and 7–140 m above the seabed. Biomass was derived by expanding the appropriate mean surface density to the area of the part. Total biomass equalled the sum of all the parts. The 0–7 m depth zone corresponded to the portion of the water column sampled by the bottom trawl.

Because of the inherent limitations in resolving targets near the seabed, data were obtained from no closer than 1.5–3.0 m off the bottom, the width of the acoustic system's tracking "bottom window." Biomass nearer the bottom was estimated by extrapolating mean volume density values obtained from between the top of the window to 7 m off the bottom.

We used a shrimp bottom trawl (Sputnik 1600) to identify targets detected near the bottom and to index the 0–7 m acoustic data. Although designed as a shrimp trawl, this net is an effective fish sampler and has been used in studies of fish populations (e.g. Bowering and Brodie 1989). The trawl had a 13-mm-mesh cod end liner, a headrope height of about 7 m, and a mouth opening width of about 22 m. Twenty-four catches were sorted and the amounts (kilograms) of each major species were recorded. Catches were weighted to a 30-min tow (towing speed = 4.8 km·h^{-1}) and were converted with a standard swept area of 54 185 m^2, based on trawl mouth dimensions. Swept area estimates of shrimp abundance and biomass were derived with the STRAP computer program (Smith and Somerton 1981) following standard protocol utilized for shrimp stock assessment. Shrimp catches were also examined for cyclic periodicity (ln catch versus time of day) using a nonlinear regression technique (SAS Institute, Cary, NC; PROC NLIN). Shrimp distribution was mapped from shrimp catch data.

Vertical Distribution of Biomass

We studied the vertical distribution of shrimp at three locations (Fig. 1) with different concentrations of shrimp and level bottom. At each location, we operated the BIONESS over (roughly) the same track every 2 h, resulting in three series of 13, 27, and 27 h. We operated the acoustic system simultaneously during series 2 and 3. The BIONESS is a multiple-net zooplankton sampler with a remote control net opening/closing capability. It was equipped with nine 1-m^2 aperture nets (333-

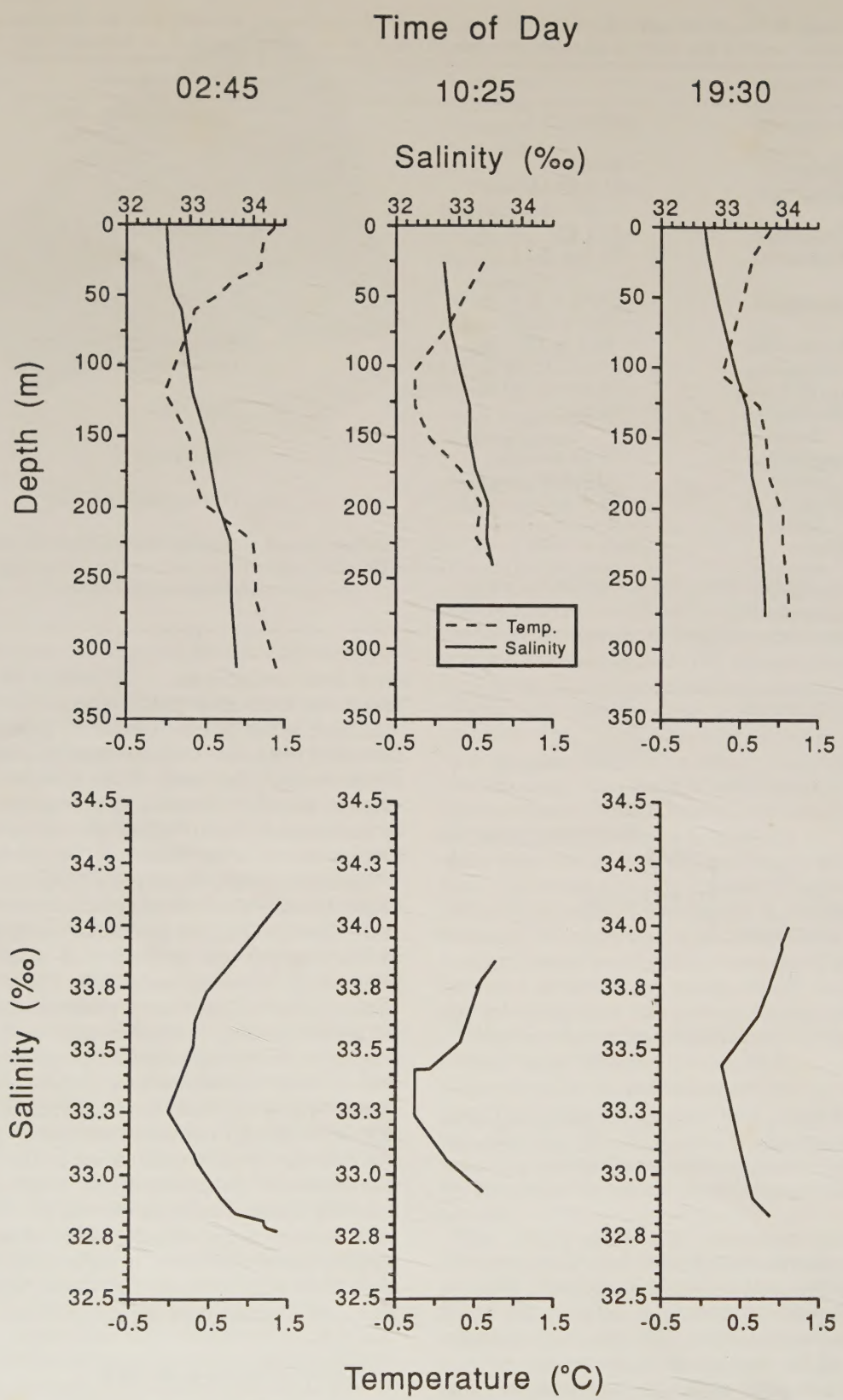

FIG. 4. Representative temperature and salinity depth profiles and TS diagrams for the study area. Data correspond to the three sampling periods depicted in Fig. 11 and 13.

and 500-μm mesh). It also had an STD for gathering depth-related salinity and temperature information. The sampler was deployed to within 10 m of the bottom and towed at about 1.6 $m \cdot s^{-1}$. As it was slowly raised, samples were taken across seven 25-m-wide layers. The last two samples covered 50–100 m vertical intervals each, depending on the remaining distance to the surface. We made a total of 34 tows (306 net samples). The presence of shrimp and other zooplankters was expressed as milligrams wet weight per cubic metre. Echo integration of the simultaneously collected acoustic data was exe-

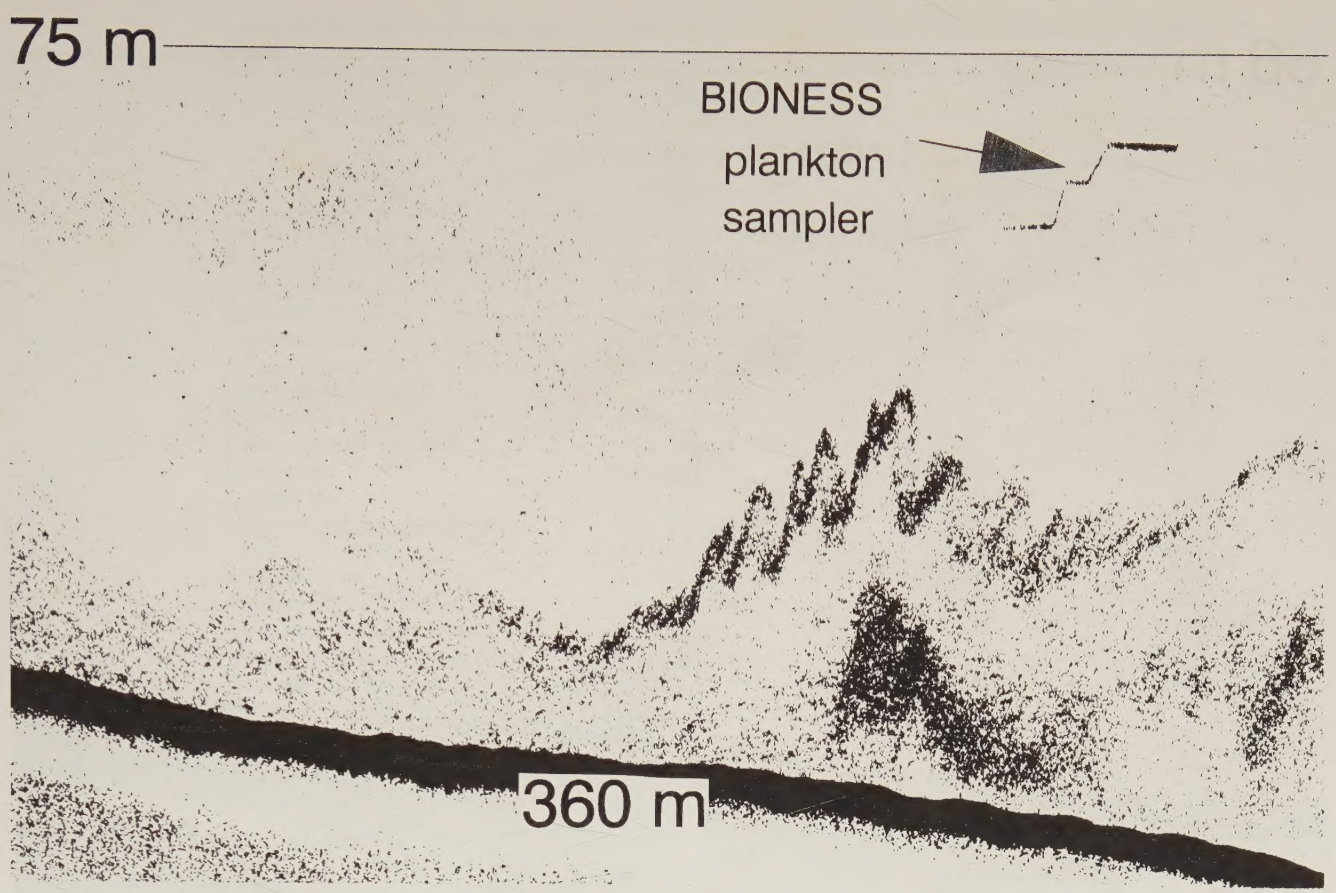

FIG. 5. Echogram indicating the displacement and coalescing of the DSL by oceanographic processes. Time of recording was 04:20–05:00; transducer depth = 75 m; bottom depth = 310–380 m. Echo from BIONESS in upper right corner.

cuted in coincident depth intervals to derive comparable estimates of biomass.

The relationships between the biomass of the 11 major groups of organisms in the BIONESS catches and the equivalent estimates of volume density were evaluated by principal components factor analysis (PCFA) (Anderson 1958). Correlations less than 0.5 between variables and principal factors were considered not meaningful. Because the water depth varied during the sampling, data were transformed to their relative position in the water column, in relation to the bottom, prior to evaluation. Net samples from depths above the transducer were omitted.

We assumed that the BIONESS is a poor sampler of fish, so we monitored the relative abundance of fish by counting the number of fish-like echoes on echograms. Counts were weighted to account for the conical shape of the acoustic beam and were considered estimates.

Results

Physical Conditions

Dawn occurred at about 05:00 and it was completely dark by about 21:30. Tidal amplitudes were increasing throughout the study period, due to autumn spring tides (Canadian Hydrographic Service 1988). Water masses were well mixed. A weak thermocline was present, oriented about a layer of colder water (centred at about 100 m) that was sandwiched between warmer surface and deeper waters (Fig. 4). The acoustics revealed that physical phenomena, such as internal waves (Fig. 5), were affecting the distribution of zooplankton.

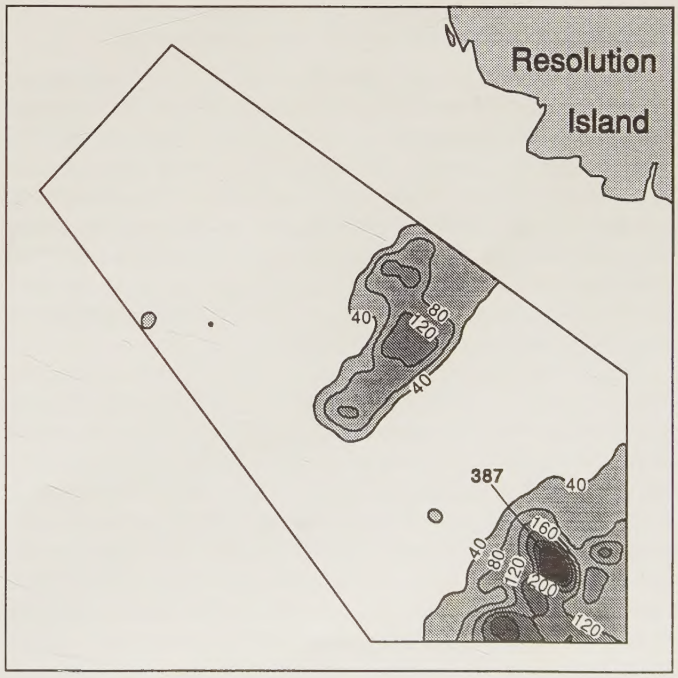

FIG. 6. Distribution of total biomass detected acoustically between the seabed and 140 m above it, within the primary study area. Isolines = 40 $g \cdot m^{-2}$.

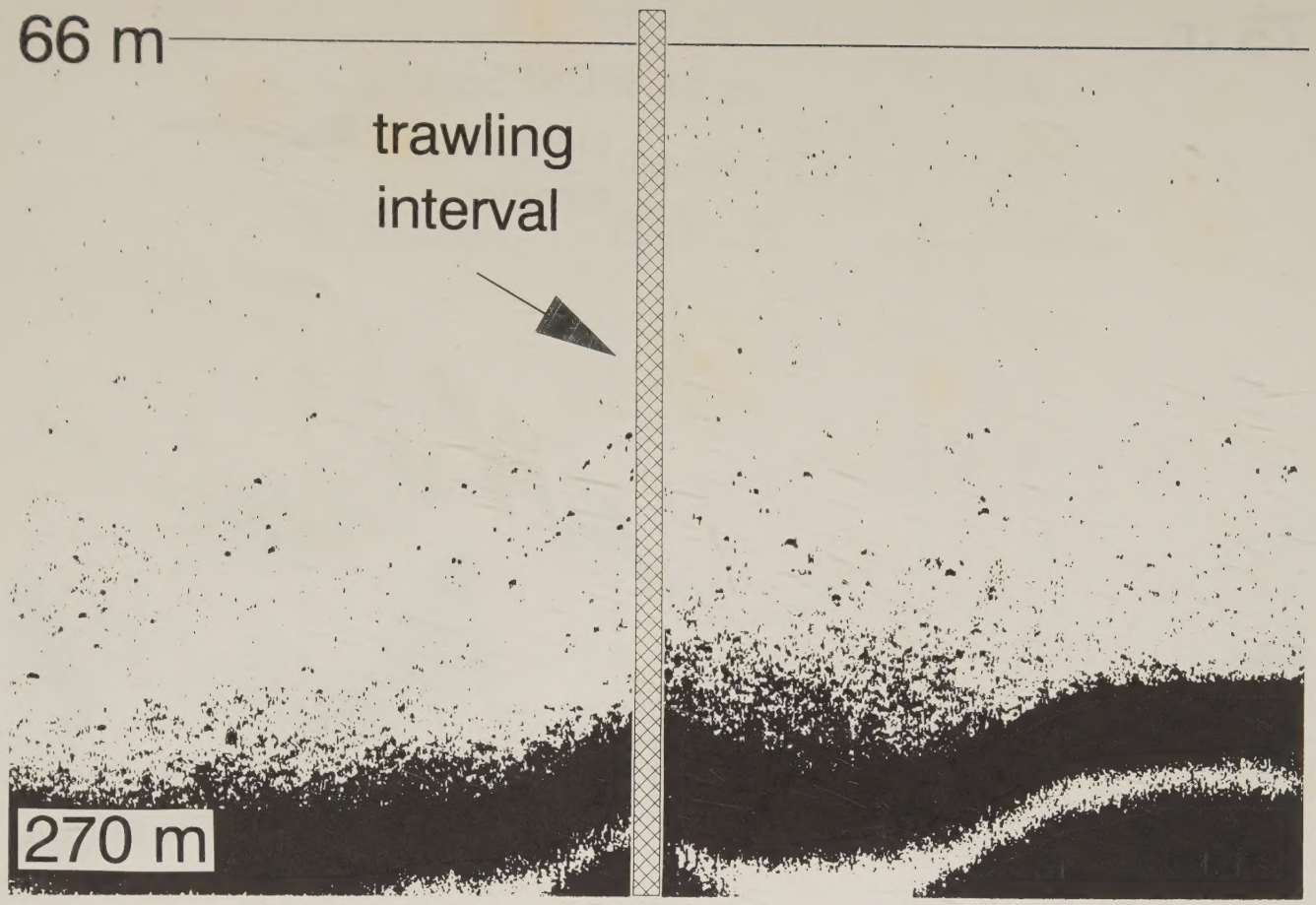

FIG. 7. Echogram obtained in the vicinity of the trench (Fig. 2) near the centre of the study area. Acoustic observation was interrupted (hatched line) to sample with a bottom trawl, which caught 467 kg of shrimp and 18 kg of fish (mostly juvenile Greenland halibut) in 30 min. The acoustic record began at 00:06, was interrupted at 00:21, resumed at 02:30, and ended at 02:46. Vessel speed was about 8.3 $km \cdot h^{-1}$.

Horizontal Distribution

Shrimp were abundant in the study area. Bottom trawl catches ranged from 0.05 to 2133.5 kg of shrimp per 30-min tow. Highest concentrations were found in the southeast corner, the deepest part of the study area (Fig. 2). A lesser concentration was located near the centre of the study area. This distribution pattern was independently corroborated by the acoustic survey (Fig. 6). Because the trawling and acoustic surveys contained information that was collected as much as 1 wk apart, the similarity of their results suggested that the horizontal distribution was stable over at least that period.

Concentrations of shrimp were apparent in the echograms (Fig. 7), but when shrimp were dispersed they were not recognizable among the community of scatterers. The amount of fish in the trawl catches was low (Table 3). For example, within all catches of more than 60 kg of shrimp, mean fish by-catch weight was 6.7%. We inferred that our acoustic estimates also contained a low proportion of fish, for the following reasons: (a) we observed no fish schools on the echograms and (b) there were fewer discreet fish echoes in the water column than near the bottom. The number of fish echoes increased only slightly in the two areas of biomass concentration. Because fish were so few, we did not attempt to account for their contribution to the acoustic data. The total acoustic biomass estimate (mean ±95% confidence interval) for the 0–7 m stratum was in close agreement with the swept area estimate of shrimp biomass determined with the bottom trawl: (1) 6367 ± 1933 t total biomass by the acoustic survey and (2) 6549 ± 4024 t of shrimp by the bottom trawl. Total acoustic biomass between 0 and 140 m was 28 900 ± 2004 t.

Periodicity within the trawl catch pattern was estimated at about 15 h (±3 h), but because of the small sample size and high residuals within the estimate, it was considered unreliable. Nevertheless, catches were highest at midday (Fig. 8). Catch composition (Table 3) suggested that fish in the central location were primarily Arctic cod (*Boreogadus saida*). Those in the southeastern location were deepwater redfish (*Sebastes mentella*) and juvenile Greenland halibut (*Reinhardtius hippoglossoides*).

Vertical Distribution

On a weight-specific basis, shrimp were the most abundant macrozooplankter in the BIONESS catches. Densities up to 304.8 $mg \cdot m^{-3}$ were observed in a single BIONESS sample (20:00, net depth = 175–200 m, water depth = 280 m). Average densities for the various depth ranges sampled by each net ranged between 3.4 and 30.9 $mg \cdot m^{-3}$ for all BIONESS series.

Shrimp densities derived from BIONESS catches were often less than corresponding total acoustic densities by about a factor of 10. However, the PCFA indicated correspondence between the net catches and echo integration. In the analysis, the primary and secondary principal factors were extracted and their corresponding correlation with each variable (species and acoustic density as milligrams per cubic metre) was examined

TABLE 3. Fish and invertebrate species caught by the bottom trawl and the BIONESS during the study period.

Bottom trawl			
		Mean catch	
Species	Common name	Weight (kg)	Var
Pandalus montagui	Striped pink shrimp	686.0	662 890.1
Reinhardtius hippoglossoides	Greenland halibut	5.61	51.17
Boreogadus saida	Arctic cod	4.4	46.0
Sebastes mentella	Deepwater redfish	3.65	59.11
Triglops nybelini	Nybelin's sculpin	2.87	126.93
Agonus decagonus	Northern alligatorfish	2.78	132.82
Lumpenus maculatus	Daubed shanny	1.51	49.19
Raja radiata	Thorny skate	1.5	4.5
Eumicrotremus spinosus	Spiny lumpsucker	1.35	2.84
Liparis sp.	Sea snail	0.68	0.71
Lycodes sp.	Eelpout	0.59	0.71
Hippoglossoides platessoides	American plaice	0.32	0.8
Triglops murrayi	Mailed sculpin	0.24	0.26
Artediellus sp.	Hookear sculpin	0.06	0.04
Myoxocephalus quadricornis	Fourhorn sculpin	0.04	0.04
Lumpenus lumpretaeformis	Snakeblenny	0.04	0.04
Myoxocephalus scorpius	Shorthorn sculpin	0.03	0.02
Icelus spatula	Spatulate sculpin	0.03	0.01
Notolepis rissoi kroyeri	Scaled lancetfish	0.03	>0
Myctophidae	Lanternfish	0.02	>0
Gaidropsarus ensis	Threebeard rockling	0.02	0.01
Lebbeus polaris	Cephalopod	0.01	>0
Gymnelus viridis	Green ocean pout	0.01	>0
Mallotus villosus	Capelin	>0	>0
Cottunculus microps	Polar sculpin	>0	>0
Aspidophoroides monopterygius	Common alligatorfish	>0	>0

BIONESS (11 most common species, alphabetically)

Aglantha digitale (cnidarian)
Calanus glacialis (calanoid copepod)
Calanus hyperboreus (calanoid copepod)
Clione limacina (pteropod)
Eukrohnia hamata (chaetognath)
Mysis polaris (mysid)
Metherytrops robusta (mysid)
Pandalus montagui (decapod)
Spiratella helicina (pteropod)
Themisto compressa (hyperiid amphipod)
Thysanoessa inermis (euphausiid)

Other: Cephalopoda, Chaetognatha, Cnidaria, Copepoda, Ctenophora, Euphausiacea, Gammaridea, Hyperiidea, Mysidacea, Ostracoda, Pisces (larvae), and Polychaeta

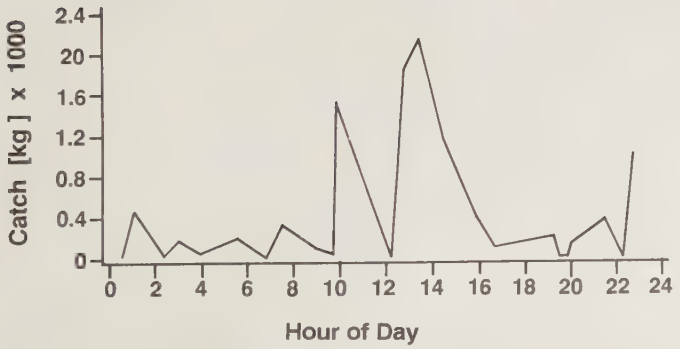

FIG. 8. Hourly bottom trawl shrimp catches within the pentagonal study area. Data were collected over several days and have been aggregated by time of day.

within the upper, middle, and bottom thirds of the ensonified portion of the water column (Fig. 9). Within the upper two thirds, acoustic and shrimp density (and that of other macrozooplankters) had high correlation ($r > 0.5$) with one of the factors, implying a relation between the variables. Within the bottom third, although the shrimp and acoustic density showed relation to each other, neither had a meaningful correlation with either principle factor ($r < 0.3$). This deviation was assumed to be a result of the influence of three divergent phenomena. First, echoes from larger fish were undoubtedly within the acoustic signal from the lower third of the water column. Second, the BIONESS did not capture fish beyond the larval stage, except for a few myctophids; their contribution to the acoustic data was judged to be insignificant. Third, the bottom portion was least effectively sampled by the oblique sampling path of the BIONESS. Accordingly, the PCFA found the strongest relationship between the net catches of macrozooplankters (e.g. shrimp, mysids, euphausiids) and the echo integration within the upper two thirds of the water column. Correlations for all variables (excluding copepod density) throughout the ensonified water column were low beyond the first two factors.

The range of average concentrations of other zooplankters within each depth interval sampled by the BIONESS varied by taxonomic group. Copepods (28–89 $mg \cdot m^{-3}$) and chaetognaths (0.4–5.0 $mg \cdot m^{-3}$) were common over the entire water

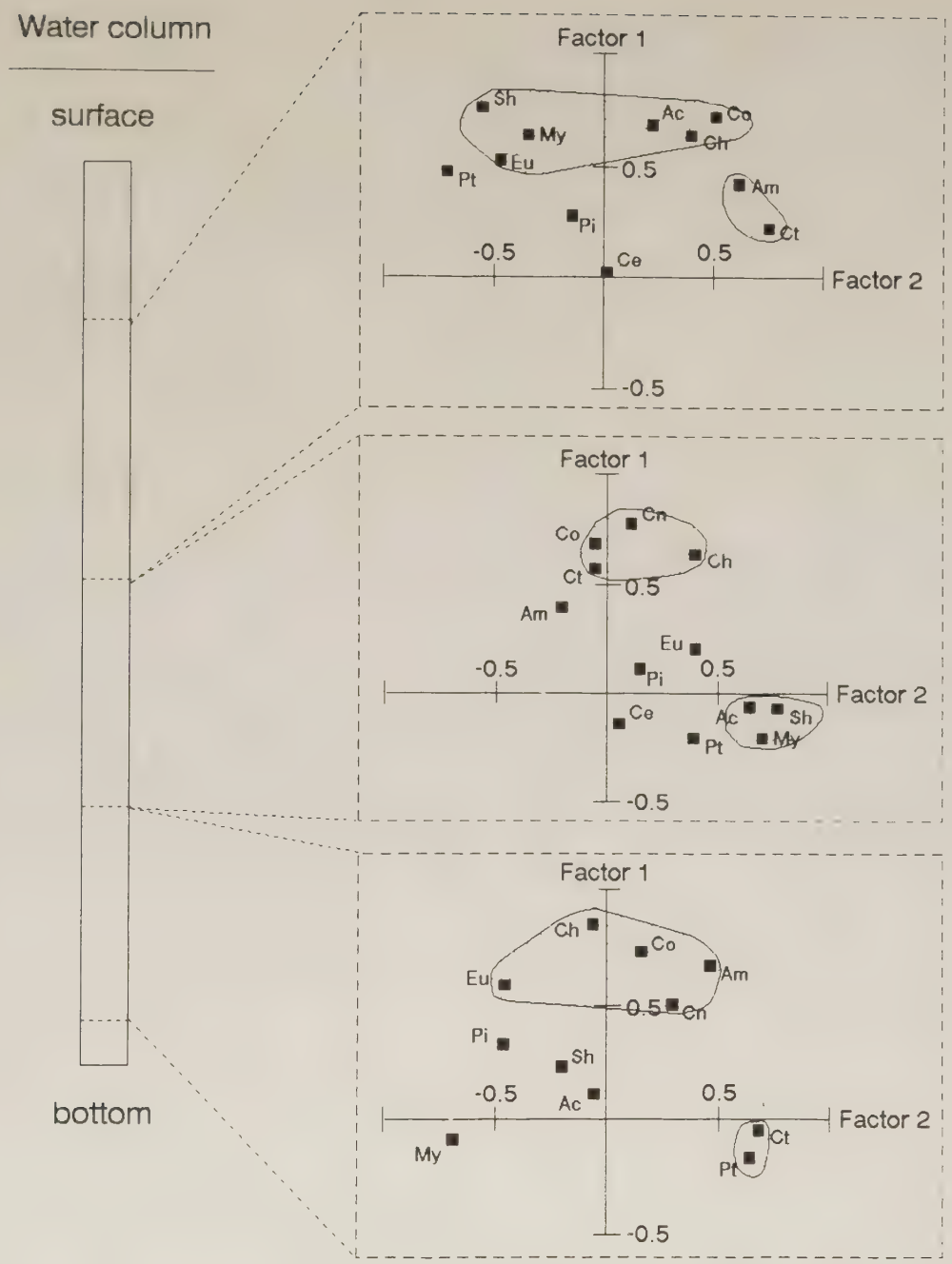

FIG. 9. Relations of the correlations between the results of echo integration, the catches of the BIONESS plankton sampler, and the primary and secondary coordinate axes by PCFA. Data points within shaded areas are considered related. Only the ensonified portion of the water column (by thirds) was evaluated. Ac = acoustics, Am = amphipods, Ce = cephalopods, Ch = chaetognaths, Cn = cnidarians, Co = copepods, Ct = ctenophores, Eu = euphausiids, My = mysids, Pi = pices, Pt = pteropods, and Sh = shrimp.

column. Concentrations of amphipods (3–23 mg·m^{-3}; to 200 m), pteropods (0.01–2.5 mg·m^{-3}; to 100 m), and cephalopods (0.0–1.3 mg·m^{-3}; to 125 m) were prevalent nearer the surface. Mysids (0.0–0.4 mg·m^{-3}) were most concentrated near the bottom. Euphausiids (0.1–0.6 mg·m^{-3}) and cnidarians (0.1–0.4 mg·m^{-3}) occurred in low concentrations throughout the water column.

The amount of shrimp biomass in the water column varied according to a diel pattern. During the day, the centre of shrimp biomass (derived from BIONESS catches) was located within the bottom 20–35% of the water column (Fig. 10). Shrimp distance above the bottom increased in late afternoon (16:00), was highest between 20:00 and 06:00, and decreased in the morning. This diel pattern was mimicked by the acoustic data which also indicated that nocturnal vertical migration occasionally extended more than 200 m off the bottom (Fig. 11). The onset of migration roughly coincided with diminishing light intensity, but the timing of peak vertical displacement varied. Acoustic data indicated that the vertical ascent rate of the scattering layer was about 2.75 m·min^{-1} (Fig. 12).

Even during the nocturnal periods of maximum vertical migration, the acoustics revealed that there was considerable

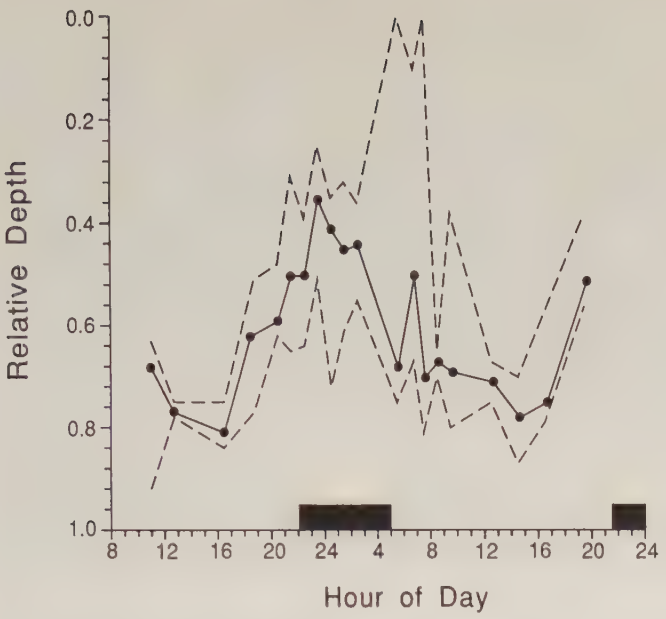

FIG. 10. Shrimp hourly depth distribution, relative to their water column position from the bottom, as the median (solid line) and quartiles (broken line) of biomass ($mg \cdot m^{-3}$) caught by the BIONESS plankton sampler. Bars indicate periods of darkness.

biomass near the bottom, an area not effectively sampled by the BIONESS. Echograms indicated fish and smaller scatterers in this region. The bottom trawl continued to catch shrimp and fish during these periods, but catches of shrimp were highest during the day (Fig. 8). Periods of high shrimp catches corresponded to the hours when catches in the BIONESS were lowest.

Shrimp descended to the bottom during early daylight hours, but not always at a predictable time (e.g. not coincident with dawn). Near midday, the acoustics generally indicated that shrimp were relatively rare in the water column (Fig. 11). However, concentrations in the order of 25 $mg \cdot m^{-3}$ were occasionally captured by the BIONESS 100 m above the bottom in the afternoon (12:00–16:00). Likewise, the water column acoustic biomass progressively increased during this time, when scattered small schools of organisms were more common.

The catches of shrimp differed significantly among BIONESS series (ANOVA, $f = 5.75$, $p = 0.004$, $n = 226$). Series 2 showed the highest concentrations (mean = 30.96 $mg \cdot m^{-3}$; SD = 56.31 $mg \cdot m^{-3}$) followed by series 1 (15.61 $mg \cdot m^{-3}$; 24.13 $mg \cdot m^{-3}$) and series 3 (10.44 $mg \cdot m^{-3}$; 21.84 $mg \cdot m^{-3}$). Concentrations also varied greatly between adjacent nets, suggesting small-scale heterogeneity in the vertical distribution. This patchiness was verified by the acoustic data which indicated that biomass was in clumps sufficiently small to be "selectively" sampled by the BIONESS (e.g. patches of biomass were also in locations not in the sampling path of the nets). For example, at 19:00 during series 2, shrimp were caught only in the middle of the water column (Fig. 13). When the BIONESS sampling path was superimposed on the echogram and echo integration "landscape" for that sampling period, the sampler was seen to have passed through a school of shrimp in midwater, but it missed a much larger mass that was deeper and associated with a bottom structure (Fig. 13a, 14).

Euphausiids and amphipods (mostly *Themisto* sp.) also exhibited diel migration (Fig. 15); chaetognaths did not. Copepods (all size classes) also did not discernably migrate, although

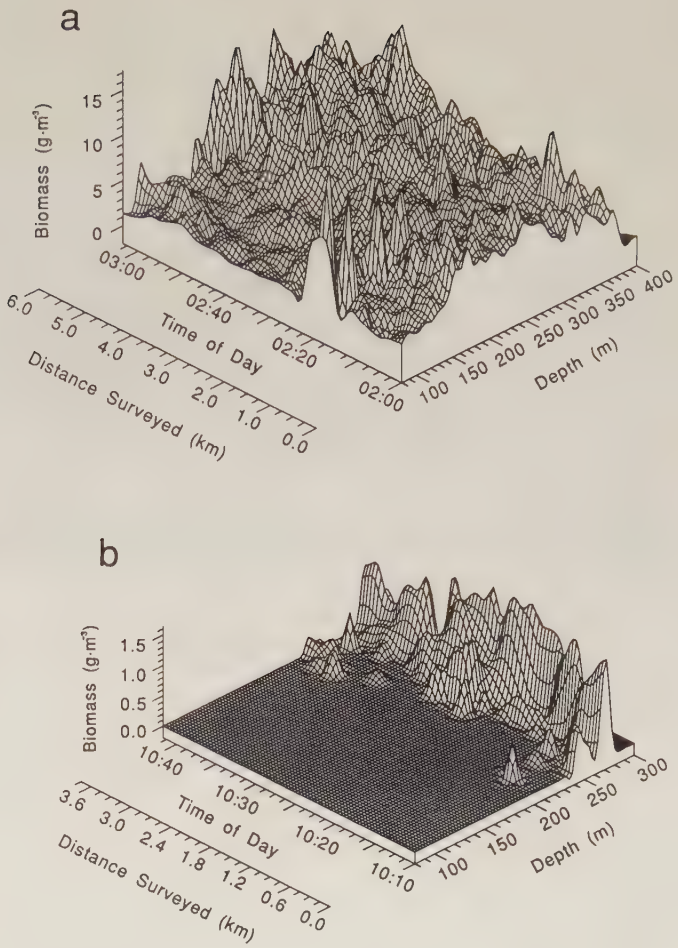

FIG. 11. Vertical distribution of total biomass detected acoustically (a) at night (02:45; water depth = 380 m) and (b) in the morning (10:25; water depth = 280 m) during BIONESS series 2. Mean BIONESS catch of shrimp in the night sample was 92.61 $mg \cdot m^{-3}$ (SD = 84.65 $mg \cdot m^{-3}$). None were caught during the day sample.

this may not have been indicative of the behaviour of the individual age groups. The PCFA correlations for copepods and chaetognaths were high for all portions of the water column, reflecting their ubiquitous vertical distribution.

Discussion

The distribution of striped pink shrimp in eastern Hudson Strait was contagious, dynamic, and followed a diel rhythm. Shrimp, and other members of the deep scattering layer (DSL), remained in two general locations within the study area during the study period but underwent significant vertical excursions at night. Bary (1967), working in coastal British Columbia, observed that the DSL ascended in four stages: (1) a gradual vertical spreading occurred about 1–2 h before sunset, (2) a period of slow ascent during sunset, (3) a rapid ascent about 1 h after sunset (at about 1–8 $m \cdot min^{-1}$), and (4) a slowing ascent and dispersion near surface. Descent followed the reverse pattern and commenced about 1–2 h before dawn. This is the same general pattern that we observed, including the rate of ascent after sunset, although variability in migration timing and daytime distribution obscured the measurement of a cyclic period from our sample of three observations. We attributed the variability to the influence of strong currents and other phenomena in the study area (Fig. 5). The horizontal displacement

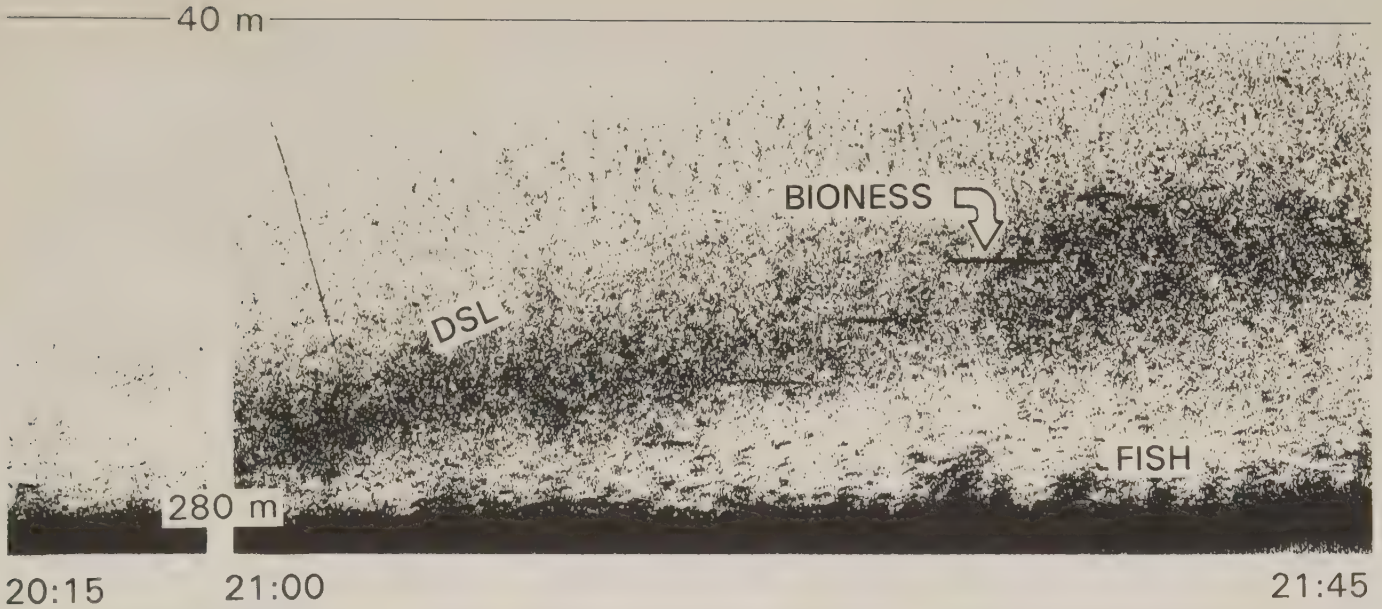

FIG. 12. Ascent of the DSL near sunset. At 20:15, scatterers were concentrated near the bottom; by 21:00, ascent had begun. Vertical migration was completed by 21:45. Mean BIONESS catch of shrimp was 58.53 mg·m ^{3} (SD = 32.05 mg·m^{-3}). Echoes from fish and the BIONESS sampler are also indicated.

FIG. 13. (a) Echogram obtained during BIONESS series 2 between 19:00 and 19:45. The probable track of BIONESS sampler is indicated by the superimposed heavy line. (b) Distribution of biomass in the water column derived from net catches during the sampling event depicted in Fig. 13a.

and vertical mixing of biological scatterers by flow processes has been observed elsewhere (Orr 1981; Haury et al. 1983) and is apparently common (Simard et al. 1986). Such phenomena are thought to be important agents of mixing (Thorpe 1978) and may contribute to the locally productive Hudson Strait ecosystem.

The vertical migration of shrimp consisted of localized concentrations that were sufficiently small to be stochastically sampled by capture gear, rather than predictably as would occur if they were distributed in larger features. This patchiness, recorded by the acoustics, was supported by the catches of the BIONESS. However, differences in concentrations observed between adjacent nets in a BIONESS series occasionally exaggerated the distribution. A more accurate interpretation of vertical distribution was possible using acoustic data.

This relationship extended into the examination of the horizontal distribution as well. The acoustic data were gathered in segments such that there were five data points collected over

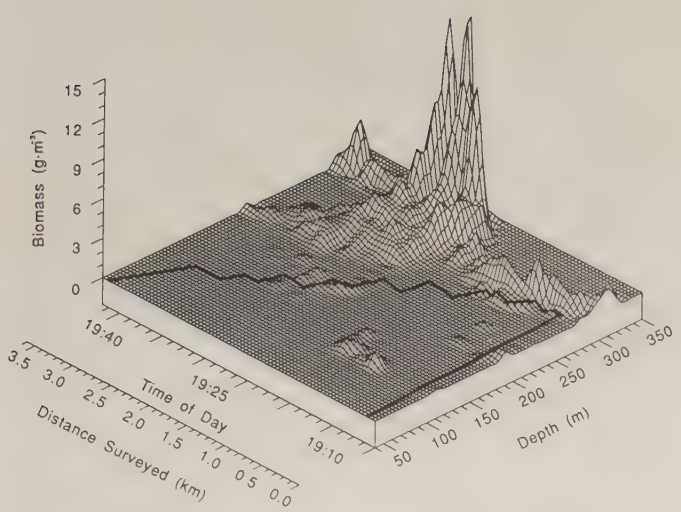

FIG. 14. Shrimp distribution derived from echo integration of acoustic data obtained during sampling described in Fig. 13. Details are the same as Fig. 13a.

the distance covered (2.4 km) during each trawl sample of our study. Accordingly, the "resolving power" was greater for the acoustics than for the trawling method. Occasionally the acoustic data revealed that some biomass concentrations were smaller than the distance covered during a sampling tow. Horizontal patchiness contributed to variability in trawl catches, but because of the homogenizing "integration effect" of a long sampling period, the ability of the trawl to describe the true nature of the distribution was diminished. Because three samples in the bottom trawl survey happened to coincide with one such concentration, the shrimp swarm was subsequently overemphasized during the trawl survey analysis (D. G. Parsons, unpubl. data).

The BIONESS also mixed samples by gathering shrimp from across a wide area and integrating the contagious elements of their distribution. Because the nine BIONESS nets were opened sequentially at each depth, the surface net sampled waters about 3 km away from the location sampled by the bottom net. This produced a distortion of the vertical distribution, obscuring this aspect that was much more easily discernible with the acoustics. This partially explains the high vertical heterogeneity observed among nets of the same BIONESS set, where strata with no shrimp occurred between strata of high shrimp concentration (or vice versa). This integration of the vertical component introduced an artifact into the data, especially when it was displayed in the traditional sense, as a vertical series with no horizontal component to the distribution (Fig. 13b). Comparing Fig. 13 and 14 emphasizes how important retaining the horizontal component is when studying the distribution of biomass in the water column.

The acoustic and bottom trawl estimates of shrimp biomass were similar not only for the stratified surveys, but also for specific sampling events. However, the concordance was serendipitous. The acoustic system was probably no more accurate than $\pm 50\%$, due to its "estimated" calibration, and the acoustic data were derived from a community of macrozooplankton and fish. The trawl data were sorted; all but shrimp were excluded. Furthermore, not only was the sampling efficiency of the trawl unknown, but studies elsewhere have indicated that this can be highly variable (Stolyarenko and Ivanov 1990). We have not attempted to describe a model that would reliably reproduce these results elsewhere. To do so would require the consideration of many more factors than were included here. Subsequent to this study, it was suggested that the FIBEX model we used as a basis for estimating shrimp TS required revision (Everson et al. 1990; Foote et al. 1990; Green et al. 1991; Hewitt and Demer 1991). Further work is also needed to define a more accurate value for the acoustic backscatter of pandalid shrimps.

The comparison of the acoustic estimates with those derived from bottom trawl catches also did not include an allowance for the extrapolated biomass value near the bottom (across the acoustic bottom window). Without exception, the acoustics indicated an increase in biomass nearer the bottom. If this trend continued into the first 1.5–3.0 m off of the bottom (the width of our bottom window), our estimates for the 0–7 m stratum could have underestimated biomass by about a factor of 2.

A nocturnal reduction in backscatter would be expected to occur with those species that are directional acoustic backscatterers and which change their swimming angle (from the horizontal) during specific behaviours (Foote 1980). For example, Antarctic krill (*Euphausia superba*) have been observed to swim in loops and spirals when feeding (Hamner et al. 1983). This species feeds primarily at night, when it is reported to have a lower TS (Everson 1982). Sameoto (1980) reported that another krill, *Thysanoessa raschii*, increased its vertical orientation when swimming at night. He suggested that this behaviour would also reduce the backscatter toward the surface. However, caution must be used during generalization regarding behaviour and TS. Price (1989) observed that when *T. raschii* was feeding, it assumed a more horizontal orientation and increased the linearity of its swimming direction. This behaviour would tend to increase its acoustic backscatter to a downward-oriented transducer, the opposite effect of that precicted for Sameoto's (1980) nocturnal observations. When all of the possible errors regarding the determination of TS are considered, it is increasingly apparent that swimming orientation must be included as a factor of considerable influence.

That the BIONESS shrimp biomass values were lower than the acoustic estimates perhaps reflected the limitations of this device for capturing larger organisms. Mean shrimp density within BIONESS samples obtained as close as 12 m from the bottom ranged up to about 5.4 shrimp·1000 m^{-3}. This value is similar to that reported by Kikuchi and Omori (1985) for pelagic oceanic shrimps (Penaeidae and Caridea) and is not typical of densities common on commerical pandalid shrimp fishing grounds. For example, the density derived from the swept area abundance estimate of this study was about 260 shrimp·1000 m^{-3}. If shrimp had been less abundant in our study area, it is uncertain whether the BIONESS catches (or the acoustic data) would have so patently revealed diel migration.

Although copepods were frequently the dominant member of the zooplankton community, there was little evidence that they were components of the 120-kHz acoustic backscatter signal, a finding also reported elsewhere (Sameoto 1980). But the lack of correspondence between the acoustic signal and certain zooplankters may have been influenced by our correction for biological noise. The noise signal was considered to be the generally constant, nonzero background signal within the acoustic data. The BIONESS detected a marked diel vertical migration of shrimp, euphausiids, mysids, and amphipods, which was reflected within the acoustic data. There was little evidence of

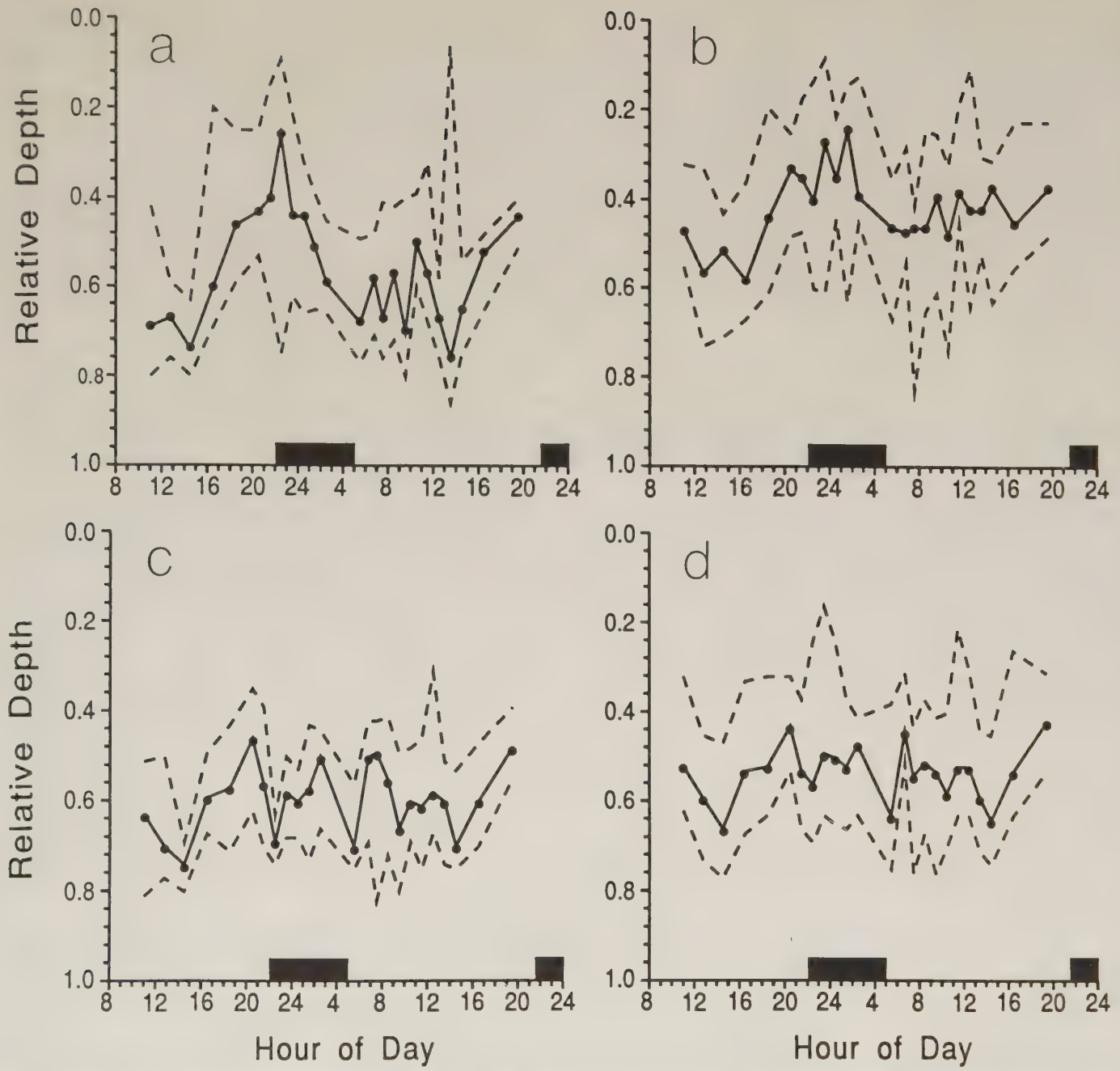

FIG. 15. Hourly depth distribution of the median (solid line) and quartiles (broken line) of the biomass of four zooplankters caught by the BIONESS. (a) Euphausiids; (b) amphipods; (c) chaetognaths; (d) copepods. Details are the same as in Fig. 10.

diel migration in net catches of copepods (all size/age classes combined) or other weak acoustic backscatterers, such as chaetognaths. Because the PCFA found little relationship between these latter catches and the acoustic signals, and because the noise values were almost constant for each sampling period (and catches were not), we assumed the noise to be a combined contribution of the background community. Although its specific source(s) remained unidentified, echoes from some of these weaker backscatterers were likely a part of the "noise."

Some of the highest bottom trawl catches of shrimp were made within or near a shallow "trench" that traversed the study area (Fig. 2). Echograms suggested that biomass was concentrated there in a manner similar to that observed in the Gulf of St. Lawrence (Simard et al. 1986) and off Georges Bank (Green et al. 1988, 1989). Fishermen have long associated good catches of fish with bottom features or "edges." Concentrations of zooplankton in these places may be the reason for the high numbers of fish there as well (Greene et al. 1989).

Although our trawl data were not site specific, a diel trend in catch rate was suggested by the higher catches at noon (Fig. 8). However, the interpretation of catch trends for indications of behaviour must also consider the concomitant influence of physical factors that is sometimes observed under specific conditions. For example, Parsons and Sandeman (1981) reported a 6-h cycle in shrimp catches in Davis Strait. The specific cause for such a brief cycle was unknown. An 11.1-h semi diel cycle was detected in shrimp catches made in the Hopedale Channel (off Labrador) in 1980 (D. G. Parsons, Northwest Atlantic Fisheries Centre, P.O. Box 5667, St. John's, Nfld. A1C 5X1, unpubl. data). It has been speculated

on the one hand that these data reflected the influence of tidal effects on shrimp behaviour and trawl performance. On the other hand, a 23.1-h diel cycle was recorded near the same location 2 yr later (D. G. Parsons, unpubl. data). There was no evidence of tidal influence during this latter work or the present study, although there was unexplained variability within the data in both cases. In this study, oceanographic phenomena were considered a significant influence on zooplankton distribution (C. Hudon, DFO Halifax Research Laboratory, Halifax, N.S. B3J 2S7, unpubl. data).

Acoustically derived indications of patchy zooplankton distribution were first described during the 1970's (Pieper 1979), although direct observations of shrimp are new (this study). Possibly because of this lack of "proof," assessments of shrimp biomass traditionally ignore the possible influence of diel changes in horizontal and vertical distribution (Parsons 1984). Although the recommendation that acoustics be used to direct net sampling programs studying vertically migrating species is not new (Pieper 1979), its applicability and need remain germane to the study of shrimp biomass and abundance.

Acknowledgements

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Effect of the Outflow from the Gulf of St. Lawrence on Nova Scotia Shelf Zooplankton

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Significant differences in concentrations (per square metre) of the copepods *Calanus glacialis* and *C. hyperboreus* found between the northeastern (NE) and southwestern (SW) halves of the Scotian Shelf were apparently related to the Gulf of St. Lawrence outflow. This outflow introduces these species to the Shelf during late winter and early spring and is probably responsible for maintaining their populations on the Shelf. Deep basins on the NE half of the Shelf are also sources of breeding animals of these two species. The influence of the Gulf outflow on *C. finmarchicus* was less clear because a large population of this species was found in the basins and beyond the Shelf break. *Temora* was the only other copepod genus to show a relationship with the Gulf outflow, indicating that it is carried onto the Shelf from the Gulf. The outflow dominated the surface water of the NE Shelf over the entire year; its influence was less marked on the SW Shelf due to mixing with slope water. This mixing in turn dilutes the concentrations of the Gulf *Calanus* spp. introduced by the Nova Scotia Current. The Gulf outflow is responsible for the high zooplankton biomass concentrations on the NE Shelf in June and October.

Les écarts appréciables notés entre les concentrations (par mètre carré) en surface des copépodes *Calanus glacialis* et *C. hyperboreus* dans les parties nord-est et sud-ouest de la plate-forme néo-écossaise semblent être attribuables à l'écoulement des eaux du golfe Saint-Laurent. Cet écoulement transporte les copépodes sur la plate-forme de la fin de l'hiver au début du printemps et explique probablement le maintien de leurs populations à cet endroit. Les bassins profonds de la partie nord-est de la plate-forme servent aussi de sources de reproducteurs de ces deux espèces de copépodes. L'influence des eaux du golfe sur *C. finmarchicus* était moins nette car une importante population de ces copépodes a été décelée dans les bassins de la plate-forme et au-delà de sa limite. Le genre *Temora* est le seul autre genre de copépodes pour lequel il a été possible de montrer l'existence d'une corrélation avec l'écoulement du golfe indiquant que ces animaux sont transportés du golfe vers la plate-forme. Cet écoulement dominait les eaux de surface de la partie nord-est de la plate-forme tout au long de l'année, son influence était cependant moins marquée dans la partie sud-ouest à cause du mélange des eaux avec celles de la pente. Ce mélange diluait à son tour les concentrations des espèces du genre *Calanus* provenant du golfe et amenées par le courant de la Nouvelle-Écosse. L'écoulement en provenance du golfe explique aussi les importantes concentrations de biomasse zooplanctonique notées en juin et octobre sur la partie nord-est de la plate-forme.

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The taxonomic composition of the copepod community on the Nova Scotia Shelf has an inshore–offshore gradient that is correlated with temperature and salinity that explains only about 25% of the copepod community variance during the summer months (Tremblay and Roff 1983).

Calanus finmarchicus is the most common copepod species on the Nova Scotia Shelf during the spring and early summer, with *C. hyperboreus* and *C. glacialis* less abundant. The latter two species are considered to be northern species (Fleminger and Hulseman 1977; Conover 1988) that have breeding populations in the Gulf of St. Lawrence and in the Labrador Current. *Calanus glacialis* and *C. hyperboreus* on the Shelf are believed to be related to the cold waters from the Gulf of St. Lawrence and Labrador Current that flood the eastern half of the Shelf (Tremblay and Roff 1983). Sameoto and Herman (1990) and Herman et al. (1991) showed differences in the distribution of these species on the Scotian Shelf, with *C. hyperboreus* and *C. glacialis* concentrating on the northeastern (NE) end of the shelf, a region that has colder bottom temperatures than the southwestern (SW) half of the Shelf (Rowell et al. 1985). The SW half of the Shelf is dominated by *C. finmarchicus*, with *C. glacialis* and *C. hyperboreus* making up less than 10% of the total *Calanus* population (Sameoto and Herman 1990).

The outflow from the Gulf influences the entire NE end of the Scotian Shelf (Trites and Walton 1975; Bugden et al. 1982). The general circulation pattern in the area (Fig. 1) shows the Nova Scotia Current (NSC) flowing out of the Gulf of St. Lawrence near the coast of Cape Breton and moving southwest near the coast; a weaker flow moves southerly over the central regions of the Shelf towards the Shelf break. The Gulf Current in the Laurentian Channel moves along the west side of the Channel and carries water around the Shelf to the south slope where it meets and mixes with the Labrador Current. The Labrador current moves SW along the Shelf break and extends over the outer regions of the Scotian Shelf (Bugden et al. 1982).

The bottom temperatures on the NE half of the Shelf are colder (<6°C) then the deep water in Emerald and LaHave basins (8–11°C) (Rowell et al. 1985; Sameoto and Herman 1990; Herman et al. 1991) due to a slope water inflow. There was no evidence of slope water influencing the deep basins on the NE half of the slope.

The NSC accounts for 75% of the net annual transport along the Shelf (Drinkwater et al. 1979). The Shelf currents carry copepods in the upper 100 m southwest off the Shelf during the winter, thus reducing the spring breeding *Calanus* populations.

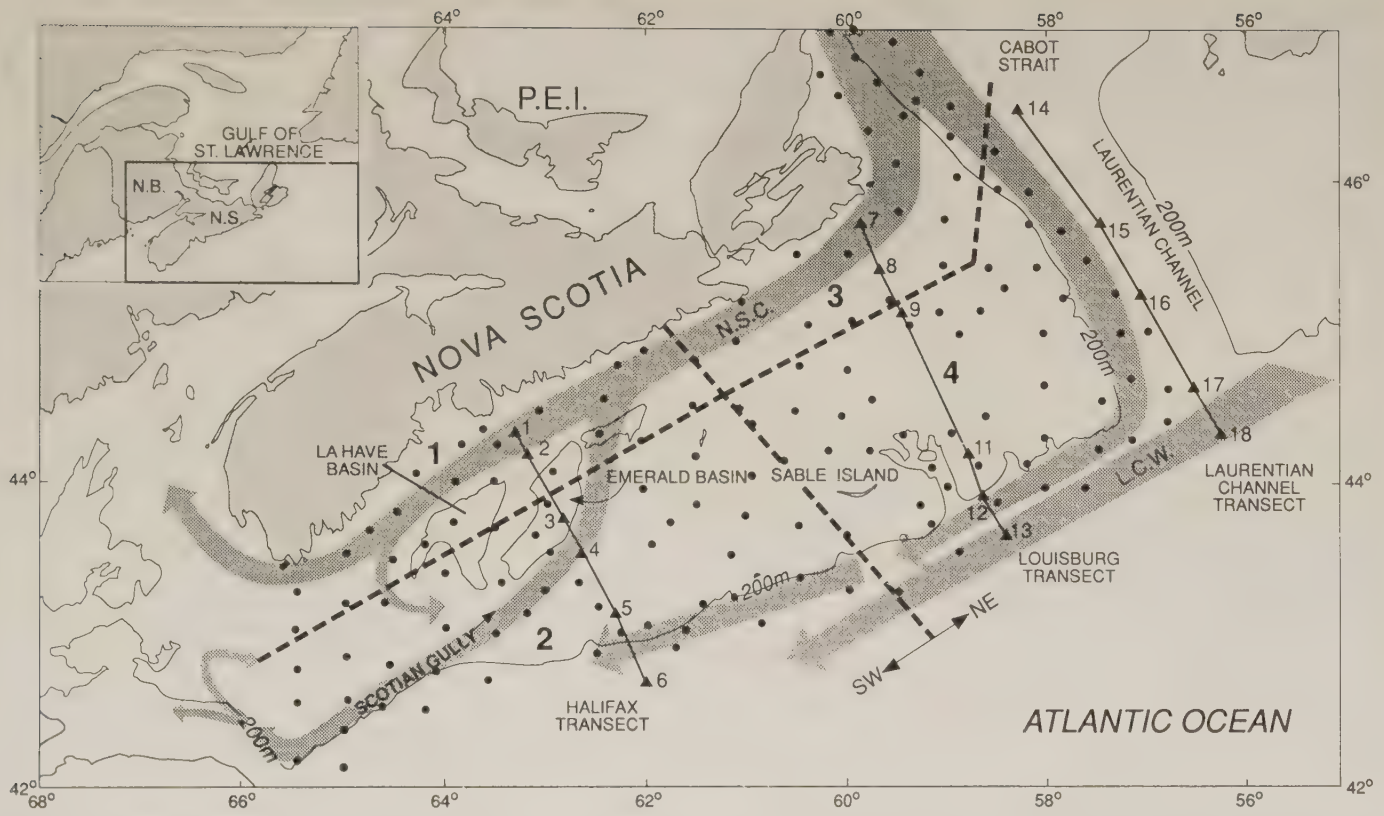

FIG. 1. Locations of SSIP stations on the Nova Scotia Shelf (solid circles), the surface circulation of the main currents on the Shelf, and the 200-m contour showing the basins and the Shelf break. The broken lines separate the Shelf into four zones, with zones 1 and 2 representing the SW half and zones 3 and 4 representing the NE half. NSC represents the Nova Scotia Current and LCW represents the slope current carrying Labrador Current water. Stations 1–6 are BIONESS stations on the Halifax transect, 7–13 are BIONESS stations on the Louisbourg transect, and 14–18 are BIONESS stations on the Laurentian Channel transect. The width of the arrows showing positions of currents is not related to the magnitude of the currents.

There are three likely sources of breeding animals on the Scotian Shelf in the late winter and spring. The first is the outflow from Gulf of St. Lawrence which has large populations of all three species of *Calanus* throughout the year (Runge and Simard 1990; De Lafontaine et al. 1991; Head 1992). The second source is the deep basins (>200 m) on the Shelf, which represent about 5% of the total Shelf area. Sameoto and Herman (1990) and Herman et al. (1991) showed that basins deeper than 200 m had large concentrations of the three species of *Calanus* in the fall. The third and least important source is the slope water beyond the Shelf break. This region has large populations of *C. finmarchicus* and *C. hyperboreus* at depths greater than 400 m during the fall and spring (Lewis and Sameoto 1988).

O'Boyle et al. (1984) showed that the eastern end of the Scotian Shelf in May had the highest zooplankton biomass (millilitres per cubic metre) on the Shelf, suggesting an influence from the Gulf of St. Lawrence. Herman et al. (1991) reported the highest concentrations of *C. finmarchicus*, *C. glacialis*, and *C. hyperboreus* in June near the coast and in the mouth of the Laurentian Channel, supporting the idea that the NSC is a major source of *Calanus* spp. on the Scotian Shelf. Estimates of zooplankton transport by the NSC (Herman et al. 1991) have indicated that approximately 50% of the Shelf populations of *Calanus* originate here, while most of the remaining animals originate from overwintering populations in the deep central basins.

This study examines the influence of the Gulf of St. Lawrence outflow on the major Shelf copepod genera during a year and on the differences in the copepod community between the NE and SW halves of the Shelf that may have resulted from this outflow. In addition, the importance of the Gulf outflow on populations of *C. finmarchicus*, *C. glacialis*, and *C. hyperboreus* is investigated to verify the hypothesis that the transport from the Gulf via the NSC carries the major part of the breeding stock of these species to the Scotian Shelf in early spring.

Methods

Zooplankton data from two sources were used for this study: Scotian Shelf Ichthyoplankton (SSIP) survey data and transect data.

SSIP Data

Zooplankton samples were collected at different months for a number of years (O'Boyle et al. 1984). They were collected with a 0.75-m-diameter, 0.333-mm-mesh bongo net towed obliquely to a maximum depth of 200 m. The species identification of *Calanus* spp. did not differentiate between *C. finmarchicus* and *C. glacialis*; therefore the numbers from the SSIP data classified as *C. finmarchicus* included both species and are identified as *C. finmarchicus* + *glacialis*. The total zooplankton biomass measured during the SSIP cruises is expressed as settled volumes (millilitres per square metre).

The SSIP data provided excellent synoptic coverage of zooplankton abundance on the Shelf between 0 and 100 m depth. Information on the vertical structure of zooplankton distributions was obtained by fine-scale vertical sampling using a multiple opening and closing net and a Batfish mounted with an optical zooplankton counter (Herman 1988; Herman et al. 1991; Sameoto and Herman 1990). Surface chlorophyll concentrations were measured on most of the zooplankton stations during the SSIP surveys using the fluorometric technique of Yentsch and Menzel (1963).

The Shelf was divided into four zones (Fig. 1). Zones 1 and 3 contained the NSC and zones 1 and 2 were areas with warmer bottom temperatures than zones 3 and 4. The SSIP data for *Calanus* and *Temora* were classified into groups representing the four zones. The dividing line between the NE and SW halves of the Shelf was west of Sable Island (Fig. 1) and was based on the difference in bottom temperatures (Rowell et al. 1985). All SSIP copepod data were transformed to $\log_{10}(x + 1)$ values. The nonparametric Mann–Whitney test on two independent samples (Conover 1971) was used to compare concentration differences in zooplankton biomass, copepod genera, and species between the two halves of the Shelf for each period sampled. Measurements of surface temperature, salinity, and chlorophyll were compared between the NE and SW halves of Shelf using the Mann–Whitney test and were also correlated with copepod concentrations using the nonparametric Spearmans coefficient of rank correlation (Conover 1971). *Calanus* and *Temora* concentrations were compared between pairs of each of the four zones using the Mann–Whitney test.

Transect Data

A survey was conducted on the Shelf and slope along three transects during a study on vertical and horizontal distributions of copepods on the Nova Scotia Shelf during April 1984 plus June and October 1985. The locations of the three transects, called Laurentian Channel, Louisbourg, and Halifax (Fig. 1), were selected to represent the different regions of the Shelf influenced by the NSC and the Gulf of St. Lawrence outflow. Mesozooplankton samples were taken with the Bedford Institute of Oceanography net and environment sensing system (BIONESS) (Sameoto et al. 1980), an opening and closing net sampler with 10 nets of 1-m^2 mouth opening, each with a mesh size of 243 μm. The BIONESS also continuously measured temperature and salinity during the tows by a Guildline Instruments digital conductivity, temperature, and depth instrument (CTD). The flow through the nets was monitored with external and internal flowmeters. The BIONESS was towed at a speed of 3 knots as it was slowly lowered along an oblique path to the desired depth where the nets were opened and closed on command from the ship. Each net filtered between 30 and 150 m^3 of water for each sample. Samples were taken within each 5–10 m depth stratum in the region from the surface to the thermocline and at approximately 10-m intervals below the thermocline. Samples were taken to within 10 m of the bottom on the Shelf and to a maximum depth of 1400 m on the slope.

Zooplankton samples were preserved in a 5% formalin seawater solution buffered with sodium borate and strontium chloride. All samples were sorted for macrozooplankton (animals >1 cm in length) and ichthyoplankton; these were removed, identified, and weighed wet after the excess water was removed by blotting. The remaining mesozooplankton in each sample were filtered over a 50-μm screen under vacuum until the water ceased to drip from the filter to remove the water and then weighed to obtain a wet biomass. The sample was split to obtain a subsample of about 400 animals using a Motoda splitter (Motoda 1959); these animals were identified to species and the *Calanus* spp. staged.

Results

Physical Parameters

The Gulf of St. Lawrence outflow was clearly delineated by salinities <31‰ on the eastern end of the Shelf from April to November (Fig. 2) and by salinities <32‰ (Petrie 1990) in February. The mixed layer depth was about 25 m (Fig. 3). The surface temperatures, salinity, and chlorophyll concentrations were compared between the NE and SW halves of the Shelf for 7 mo (Table 1). The temperatures were significantly warmer on the SW Shelf for all months except August and September. During August and September, there were no significant differences in temperature between the halves. The salinity was significantly higher on the SW half during all months except April when there was no significant difference between halves.

The chlorophyll concentrations were significantly higher on the NE half during February and April but lower during September and November (Fig. 2). No significant difference was found during the other months (Table 1). Chlorophyll was positively correlated with salinity on the SW Shelf in September and with salinity on both halves of the Shelf during November.

SSIP Copepod Distributions

The integrated concentrations (numbers per square metre) of *C. finmarchicus + glacialis* and *C. hyperboreus* on the NE and SW halves of the Scotian Shelf showed temporal patterns over a year (Fig. 4). *Calanus hyperboreus* concentrations were significantly higher on the NE Shelf for all months except August when sampling coverage was poor whereas *C. finmarchicus + glacialis* concentrations were significantly higher on the SW Shelf during April and June, with no significant difference in August. During February, May, September, and November the concentrations were significantly higher on the NE half (Table 2). The highest concentrations of *C. hyperboreus* on the NE half of the Shelf were found on the most easterly region whereas the *C. finmarchicus + glacialis* populations were more uniformly distributed over the two halves of the Shelf (Fig. 4).

Concentrations of *C. finmarchicus + glacialis* were significantly higher in zone 3 than in the other zones during June and November, and *C. hyperboreus* concentrations were significantly higher in zone 3 during February. *Temora* concentrations were significantly higher in zones 1 and 3 (the region of the NSC) during February and April than in zones 2 and 4. The other common copepod genera, *Centropages, Metridia, Oithona, Pseudocalanus, Paracalanus*, and *Clausocalanus*, had either significantly higher concentrations on the SW Shelf or were not significantly different between halves of the Shelf during various months (Table 3).

Relationships among Temperature, Salinity, Surface Chlorophyll, and Copepods

Centropages was the only genus that showed a consistent positive correlation with temperature (Table 4). *Calanus hyperboreus* and *Temora* were always negatively correlated

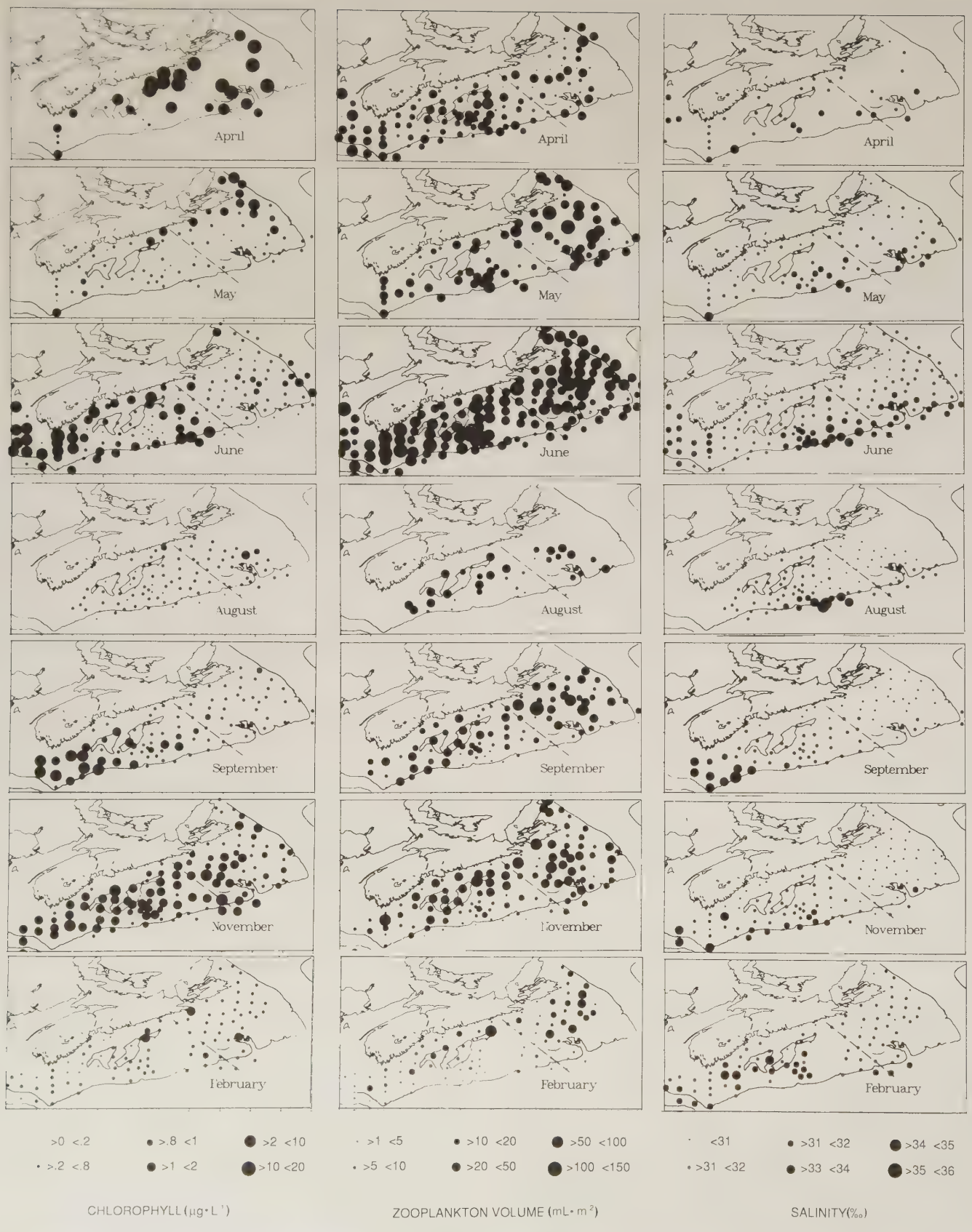

FIG. 2. Chlorophyll, zooplankton biomass, and salinity values collected during the SSIP surveys for different months. Chlorophyll data and salinity are surface values and zooplankton data represent the entire water column to a maximum depth of 200 m.

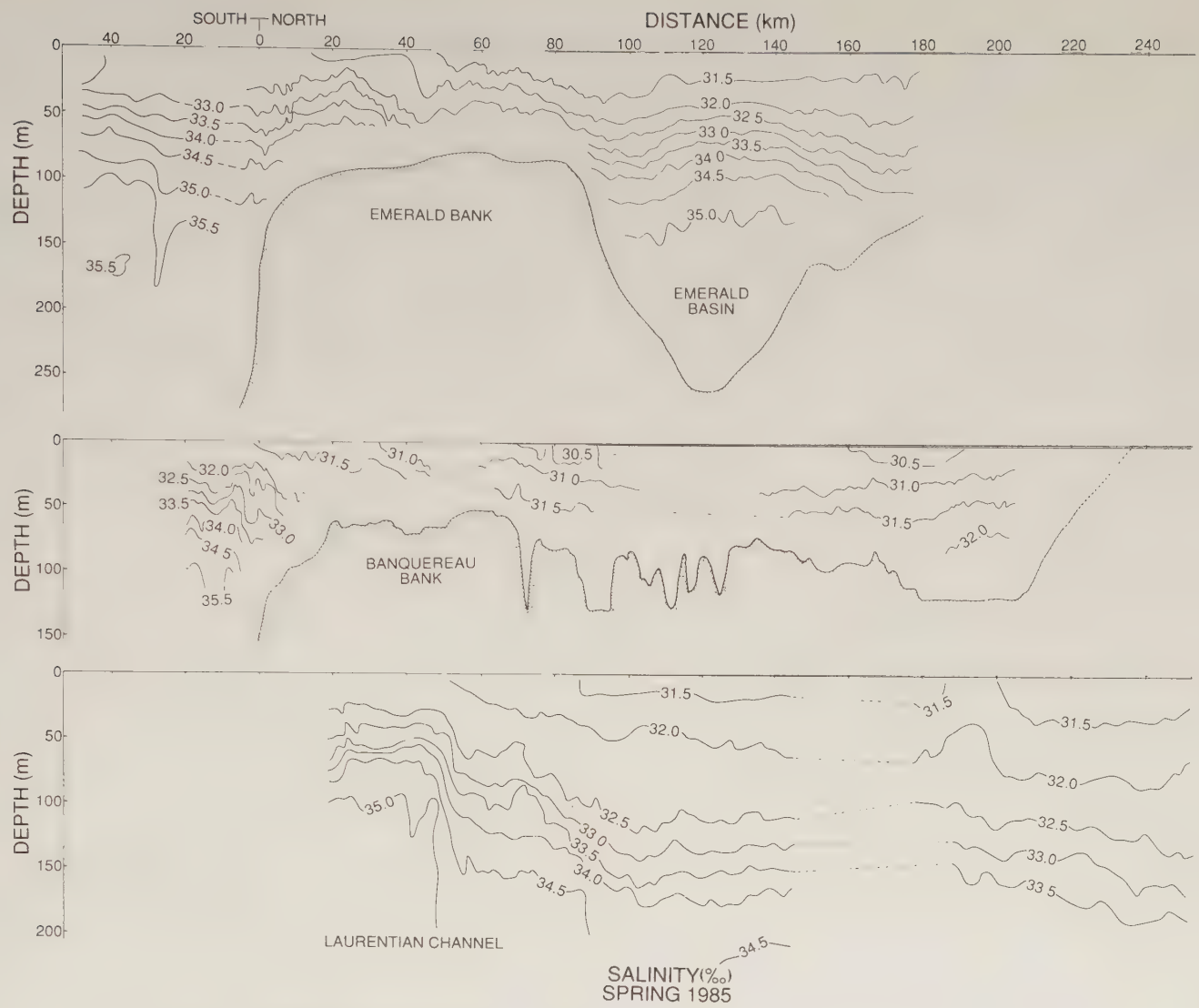

FIG. 3. Salinity/depth contours along the three transects taken with the Batfish during June 1985.

TABLE 1. Mean values for temperature, salinity, surface chlorophyll, and arithmetic mean of total zooplankton biomass for the SW and NE halves of the Scotian Shelf plus the results of Spearman rank correlation coefficients between the two halves for each variable. *SW half significantly higher (P = 0.05); **NE half significantly higher (P = 0.05).

	Temperature		Salinity		Chlorophyll		Zooplankton biomass ($mL \cdot m^{-2}$)	
Month	SW	NE	SW	NE	SW	NE	SW	NE
February	2.66*	0.29	31.85*	31.35	0.35	0.53**	8.9	11.8
April	3.91*	0.99	31.81	31.58	4.5	9.6**	30.2*	17.5
May	9.75*	5.65	31.77*	31.35	0.52	0.68	32.7	43.9**
June	10.6*	6.16	32.18*	31.71			41.1	39.9
August	18.5	17.56	31.34*	30.71	0.26	0.44	13.5	13.8
September	14.8	14.95	31.75*	30.29	1.17*	0.51	19.1	26.5**
November	9.97*	9.11	31.54*	30.70	1.17*	0.95	8.8	11.8**

with salinity, and *C. finmarchicus* + *glacialis* was negatively correlated with salinity 4 out of 5 mo.

Metridia and *Temora* had a significant negative correlation with surface chlorophyll during February, and *C. finmarchicus* + *glacialis* had a negative correlation with chlorophyll in April. From August to November, all the significant correlations between the different genera and chlorophyll were positive; only *C. hyperboreus* did not show a significant relationship with surface chlorophyll (Table 4).

Chlorophyll concentrations were higher on the NE Shelf during April and May. The September to November chlorophyll levels increased in concentration from west to east (Fig. 2).

FIG. 4. Concentrations of *C. hyperboreus* and *C. finmarchicus* + *glacialis* on the SSIP stations for different months. Values are from the entire water column to a maximum depth of 200 m.

TABLE 2. Results of the Mann–Whitney test for populations of *C. finmarchicus* + *hyperboreus* (m^{-2}) from the SW and NE halves of the Scotian Shelf. The hypothesis tested is that the NE population equals the SW population. *SW population significantly larger ($P = 0.05$); **NE population significantly larger ($P = 0.05$). Arithmetic mean, logarithmic variation (V), and 95% confidence limits (CL) calculated as a percentage of the means for the different months are given.

	C. finmarchicus + *glacialis*						*C. hyperboreus*					
	SW			NE			SW			NE		
Month	Mean	*V*	CL	Mean	*V*	CL	Mean	*V*	CL	Mean	*V*	CL
February	1 940	1.94	3 763 1 000	5 274**	1.56	8 227 3 380	10*	10.7	107 1	74	14.2	1 050 5
April	24 782*	1.09	27 012 22 735	10 773	1.14	12 281 9 450	10*	30	300 0.3	92	22	2 024 4
May	17 868	1.24	22 156 14 409	26 264**	1.29	33 880 20 359	24*	25.9	621 1	482	43.67	21 049 11
June	21 550*	3.9	84 045 5 525	892	100	89 200 9	45*	25.4	1143 2	1054	8.12	8 587 130
August	3 149	14.5	47 660 217	1 325	29.84	39 538 44	3	16	48 0.2	4	16	64 0.25
September	6 277	6.41	40 235 979	15 358*	1.62	24 879 9 480	250	12.5	350 18	984*	39.74	39 104 25
November	1 619*	9	14 571 179	10 516	2.9	32 496 3 626	5*	14	70 0.4	159	24	3 165 7

TABLE 3. Results of the Mann–Whitney test between copepod genera (other than *Calanus*) on the SW and NE halves of the Scotian Shelf. *Genera significantly different and largest population on the SW half; **genera significantly different and largest population on the NE half; NS, no significant difference between the two halves.

Month	*Centropages*	*Metridia*	*Oithona*	*Pseudocalanus*	*Pseudocalanus* plus *Clausocalanus*	*Temora*
February	*	*	*	NS	*	*
April	NS	*	NS	NS	NS	NS
May	NS	*	NS	NS	NS	NS
June	*	*	NS	*	NS	NS
August	NS	NS	NS	NS	NS	NS
September	NS	*	NS	*	*	*
November–December	*	*	*	NS	*	**

Biomass Distributions

Total zooplankton biomass (millilitres per square metre) was significantly higher on the NE Shelf during May, September, and November, but was higher on the SW Shelf in April. During June, August, and February, there was no significant difference between the two halves of the Shelf (Table 1; Fig. 2).

The pattern of total zooplankton biomass (grams per square metre) on the Laurentian (stations 14–18) and Louisbourg (stations 7–13) transects showed a sharp increase at the slope stations 18 and 13 that was not seen on slope station 6 of the Halifax transect (Fig. 5). The biomass on the slope stations was similar for all transects in June, but was lower on the Halifax transect in October. The biomass changes across these two transects for different months had similar patterns, with high values found near the coast that decreased towards the Shelf break but then increased again on the slope. The high biomass near the coast was not recorded on the Halifax transect during June or October but was found in Emerald Basin (station 3) in October. *Calanus* copepods represented between 60 and 90% of the total net zooplankton biomass on all stations.

Horizontal and Vertical Distributions of *Calanus*, 1984–85

In April, total *C. finmarchicus* concentrations were higher near the mouth of the Laurentian Channel (station 18) than near the entrance to the Gulf of St. Lawrence (station 14) whereas *C. glacialis* and *C. hyperboreus* had higher concentrations near the mouth of the Gulf. *Calanus finmarchicus* on the Louisbourg transect had a pattern similar to that shown on the Laurentian Channel, with the highest concentrations, made up of stages C5 and C6, at the slope station 13. *Calanus hyperboreus* was concentrated near the coast in the NSC (station 8) whereas *C. glacialis* was uniformly distributed across the Shelf (Fig. 6).

The concentrations of *C. finmarchicus* in June were higher at the mouth of the Laurentian Channel (station 18) than near the entrance to the Gulf, but low in the central region of the Channel (Fig. 7). The high concentrations at station 18 were due to stage C5 found at depths below 300 m, while station 14 was dominated by stages C1–C3 in the upper 50 m. The maximum concentrations of *C. glacialis* and *C. hyperboreus* occurred at station 14, with *C. glacialis* stages C2–C5 and *C. hyperboreus* stages C5 and C6 dominant. The Louisbourg transect had distributions of the three *Calanus* spp. similar to that of the Channel transect. The Halifax transect differed from the two above transects in that it had a large population of *C. finmarchicus* at stations 3 and 4 and low concentrations of the three species on the slope station (station 6).

In October, concentrations of all three species were highest in the region of the NSC on the Laurentian channel transect

TABLE 4. Results of Spearmans rank correlation coefficients tests between surface temperature, chlorophyll concentration, and different common genera of copepods on the SW and NE halves of the Scotian Shelf. +, positive correlation ($P > 0.05$); −, negative correlation ($P > 0.05$).

	February		April		May		June		August		September		November	
	SW	NE	SW	NE	SW	NE	SW	NE	SW	NE	SW	NE	SW	NE
							Temperature							
Chlorophyll		+	−			−			−					+
C. finmarchicus	−		+						−					−
C. hyperboreus		−				−								
Pseudocalanus		−				−			−			+		+
Metridia			+											
Temora	−	−		−	+	−								−
Centropages										+		+		+
							Salinity							
C. finmarchicus			+			−				−			−	−
C. hyperboreus						−		−			−	−		
Pseudocalanus						−						+		
Metridia													−	
Temora	−		−	−					−		−	−	−	−
Centropages					+									
							Chlorophyll							
C. finmarchicus			−									+	+	+
Pseudocalanus									+	+	+			
Temora		−							+	+			+	
Centropages										+			+	+
Oithona	−													
Metridia	−								+					

(Fig. 8). The Louisbourg transect had high concentrations of *C. finmarchicus* near the coast, while *C. glacialis* and *C. hyperboreus* concentrations were similar at all stations. *Calanus finmarchicus* had large concentrations of stages C1–C4 whereas *C. glacialis* and *C. hyperboreus* were dominated by stages C4 and C5. The Halifax transect had high concentrations of the three species at the Emerald Basin station (station 3) and, as in June, the concentrations of *Calanus* were low near the coast and on the slope.

Discussion

Surface salinity was consistently lower near the coast on the NE Shelf than it was near the Shelf break or on the SW Shelf. The low salinity (<31‰) near the coast during May reflected the path of the NSC. From June to November, this low-salinity water covered most of the NE Shelf. Houghton et al. (1978) reported that a mixture of Gulf of St. Lawrence surface water and subsurface (100–150 m) Cabot Strait water dominates the NE Shelf to a depth of 100 m. West of Sable Island the NSC and Cabot Strait waters were mixed with slope water at the outer edge of the Shelf and confine pure St. Lawrence water to a narrow region along the coast. The only pure slope water is found in the Emerald Basin. This mixing reduces the percentage of Gulf of St. Lawrence water on the SW Shelf to less than 50% (Houghton et al. 1978). This dilution by slope water would also dilute the concentrations of copepods carried by the Gulf water and may partially explain the reduced concentrations of *C. glacialis* and *C. hyperboreus* on the SW Shelf. However, this dilution effect does not explain the approximate 80% drop in concentration of *C. glacialis*. Sameoto and Herman (1990) observed that during June and October, approximately 33% of the *Calanus* on the NE Shelf were *C. glacialis*; in contrast, on the SW Shelf, only about 4% were *C. glacialis*.

The distributions of *C. glacialis* and *C. hyperboreus* showed that these species are brought onto the Shelf by the NSC flowing from the Gulf of St. Lawrence, but the exact time of the year this happens is unclear. The major outflow from the Gulf occurs between March and June (Drinkwater et al. 1979). It is likely that the major influx of *C. hyperboreus* from the Gulf occurs during March and/or April. This view is supported by the *C. hyperboreus* distributions in transect surveys and by Herman et al. (1991) who observed high concentrations of *C. hyperboreus* in the NSC near the Louisbourg transect during June. During all months the highest concentrations of *C. glacialis* and *C. hyperboreus* were seen on the NE Shelf which is under the direct influence of the Gulf outflow.

Other sources of *C. glacialis* and *C. hyperboreus* on the Shelf are the deep basins, many of which are found on the NE half. Sameoto and Herman (1990) found that these basins contained large populations of stages C5 and C6 of all three species of *Calanus* during the summer and fall in approximately equal numbers. These animals likely add significantly to the spring breeding populations that are carried onto the Shelf from the Gulf.

There was no evidence that the waters over the slope contributed significantly to the populations of *C. glacialis* and *C. hyperboreus* on the Shelf. The concentrations of these copepods on the slope were low compared with those in the NSC. Also, the concentration gradients of these animals are high from the coast to the Shelf break, rather than the other way around. Some animals may reach the Shelf by being carried to the Shelf

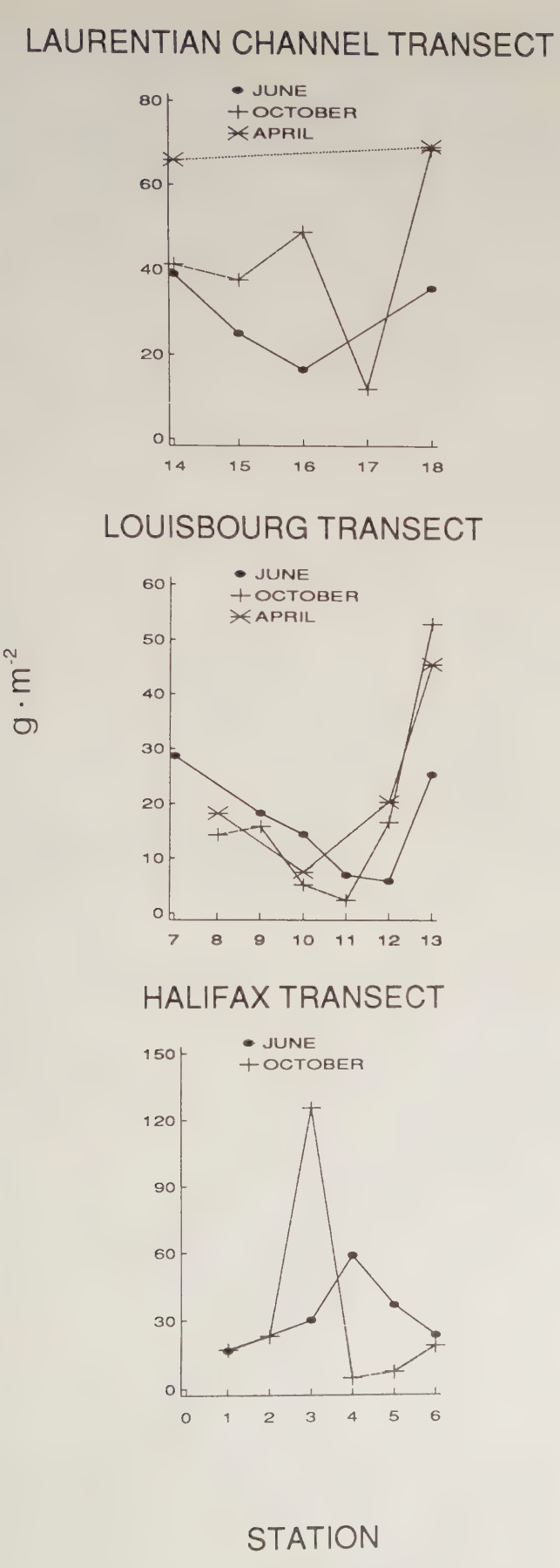

FIG. 5. Zooplankton biomass along the three BIONESS transects for different months. Note change of scale on the *y*-axis.

break region by the weaker flow of Gulf water that moves along the western edge of the Laurentian Channel. The currents in this region are not clearly understood, but evidence suggests that this circulation onto the Shelf occurs (Herman et al. 1991).

The sources of *C. finmarchicus* on the Shelf are not as clear as for the other species of *Calanus*. The SSIP data did not show a strong east to west gradient, and the transect data showed higher concentrations of *C. finmarchicus* on the outer regions of the shelf than in the NSC. This suggests either an additional off-Shelf source or more likely a concentration of animals in the surface waters near the Shelf/slope water front (Herman et al. 1981). These copepods may be Shelf animals carried by the off-Shelf currents. During the April transect survey the largest concentrations of *C. finmarchicus* on the Laurentian Channel and Louisbourg transects were on the slope stations. These animals could have been carried onto the NE Shelf as the Labrador water mixed with the Gulf water. However, the high concentrations of *C. finmarchicus* on the slope station of the Louisbourg transect may also have originated from a deep canyon to the southwest of the slope station, or from the many deep basins on this part of the Shelf. Recent data (D. D. Sameoto, unpubl. data) for example show large concentrations of *C. finmarchicus* in the deep regions of this canyon. In June and October the concentrations in the upper 100 m were higher near the coast on the Louisbourg and Laurentian transects than near the Shelf break, suggesting a link with the NSC. This pattern was not seen on the Halifax line where the NSC is weaker and where cross-Shelf mixing has had time to reduce the concentrations. The Emerald Basin contains a large population of *C. finmarchicus* below 200 m from May to the late fall that likely dominates the breeding population in early spring (Sameoto and Herman 1990). Therefore, there may be three different sources of *C. finmarchicus* on the Shelf. The first source is from the deep Shelf basins, all of which harbour large populations of stages C5 and C6 animals over the winter that migrate to the surface waters and breed in the spring (Sameoto and Herman 1990). The spring breeding population of *C. finmarchicus* on the SW Shelf likely originated principally from these basins. The second source is the NSC; the SSIP data indicated higher concentrations in this region. The third source consists of animals that migrate to the surface waters from the Labrador Current water in early spring and may be carried onto the Shelf. This last source is probably the least important of the three. The general surface circulation is off the Shelf, therefore making it difficult for slope copepods to reach the Shelf. But since the detailed seasonal circulation is not clearly understood, intrusions of surface water with copepods from the slope could flood the outer regions of the Shelf.

Calanus hyperboreus reproduces in midwinter (Conover 1967) and usually migrates to deep water in the spring as stages C3 and C4 where it remains until the next breeding period. *Calanus glacialis* appears to breed in early spring and probably has only one generation. This suggests that it begins its seasonal migration into deep water as stages C4 and C5 by May (Sameoto and Herman 1990). Therefore, animals carried out of the Gulf in winter and early spring as nauplii or early copepodites will mature to stages C4 and C5 before they are carried to the SW Shelf. It takes about 3 mo for the Gulf water by way of the NSC during spring to reach the Halifax transect in spring (Herman et al. 1991). This indicates that most of the copepods will have matured to stage C4 and begun to migrate downward and accumulate in the deep basins on the NE Shelf. Late-maturing

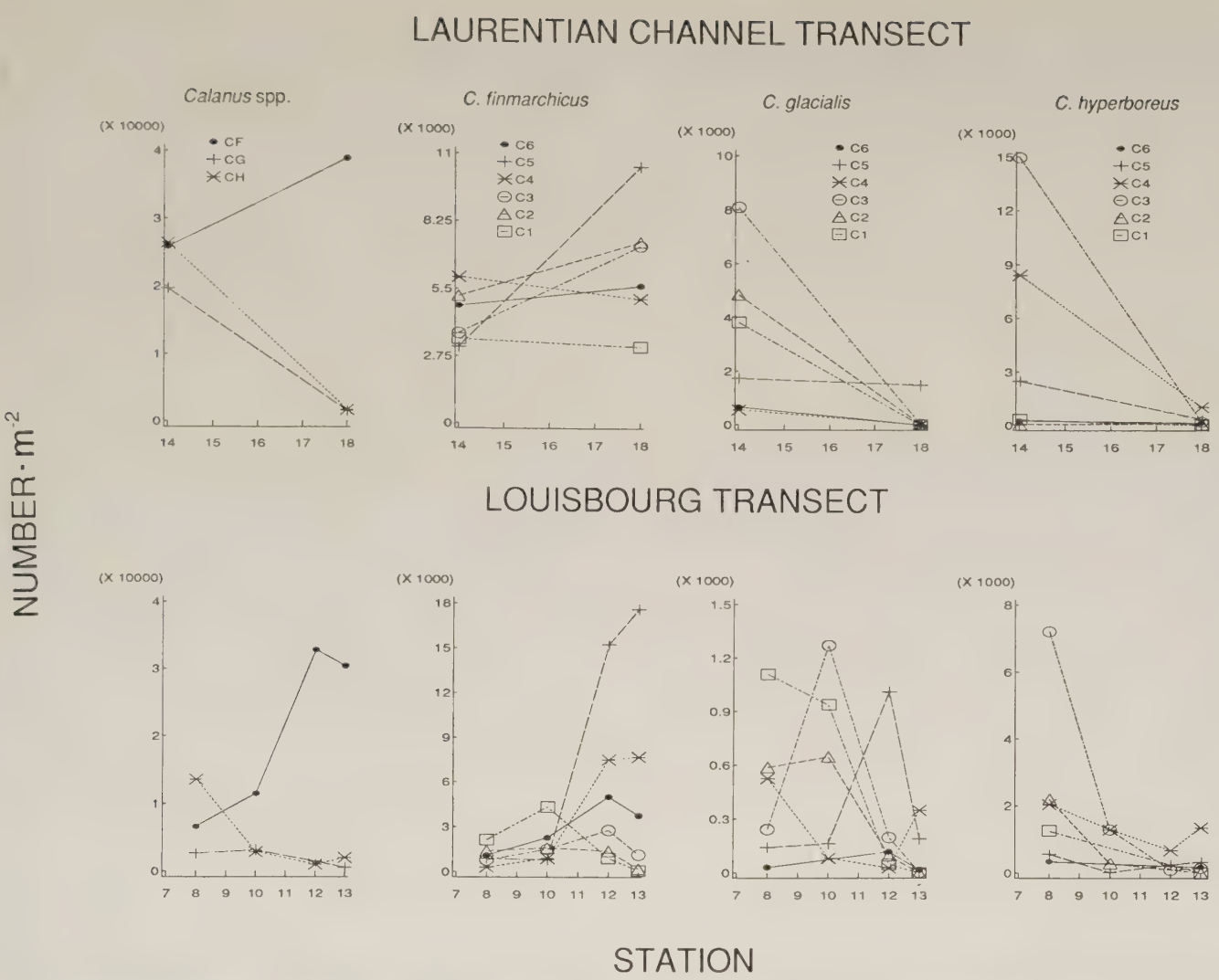

FIG. 6. Concentrations of total *C. finmarchicus* (CF), *C. glacialis* (CG), *C. hyperboreus* (CH), and various copepodite stages of each species during April 1984 on the different stations on the Laurentian Channel and Louisbourg transects. Note change of scale on the *y*-axis.

animals that are carried to the SW Shelf will accumulate in one of the two basins or be carried off the Shelf (Herman et al. 1991).

Calanus finmarchicus has at least two to three generations during the summer on the Scotian Shelf (McLaren et al. 1989; Sameoto and Herman 1990). The new generations of *C. finmarchicus* originating from Gulf animals in the NSC and from deep basin animals will intermix during the spring and summer, thereby masking evidence of a NE–SW population gradient related to the NSC. *Calanus finmarchicus* actively reproduced in October on the Louisbourg and Laurentian Channel transects, as evidenced by stage C1 and C2 animals. There was no significant population of young copepodites on the Halifax transect, indicating that the environmental conditions on the Halifax transect were different than on the other two. The factors that allowed *C. finmarchicus* to reproduce actively in the fall on the NE Shelf and not on the SW Shelf are unknown.

The negative correlations between the *Calanus* spp., *Temora*, and salinity suggested that low-salinity Gulf water and high concentrations of these copepods were related. In addition, high concentrations of these species in zone 3 during the months of high outflow from the Gulf indicated that the Gulf was a major source of these copepods. De Lafontaine et al. (1991) reported a large spring population of *Temora longicornis* in the Gulf. The other copepod genera showed no consistent correlations with salinity and were not more abundant on the NE Shelf, indicating that they probably were not influenced by the Gulf outflow.

Surface chlorophyll was higher in the Gulf water on the NE Shelf during April and May, but the copepods either were negatively correlated or showed no correlation with the chlorophyll concentrations. However, during the summer and fall, all the genera except *Oithona* showed a positive correlation with high chlorophyll concentrations on the SW Shelf. These results suggest that the Gulf outflow has little influence on Shelf copepods by way of primary production on the Shelf.

The total zooplankton biomass was generally higher in the region of the NSC on the Laurentian Channel and Louisbourg transects. In May and October the biomass was significantly higher on the NE Shelf. These data suggested that the increased population of *Calanus* spp. resulted in higher biomass concentrations and support the findings of Herman et al. (1991) who showed a link between the Gulf outflow and zooplankton biomass in these regions of the Shelf. The volume of the Gulf outflow varies year to year (Bugden et al. 1982). This variation in outflow likely affects the size of the population of *Calanus* spp. introduced to the Nova Scotia Shelf and that in turn may affect the overall zooplankton production and biomass on the

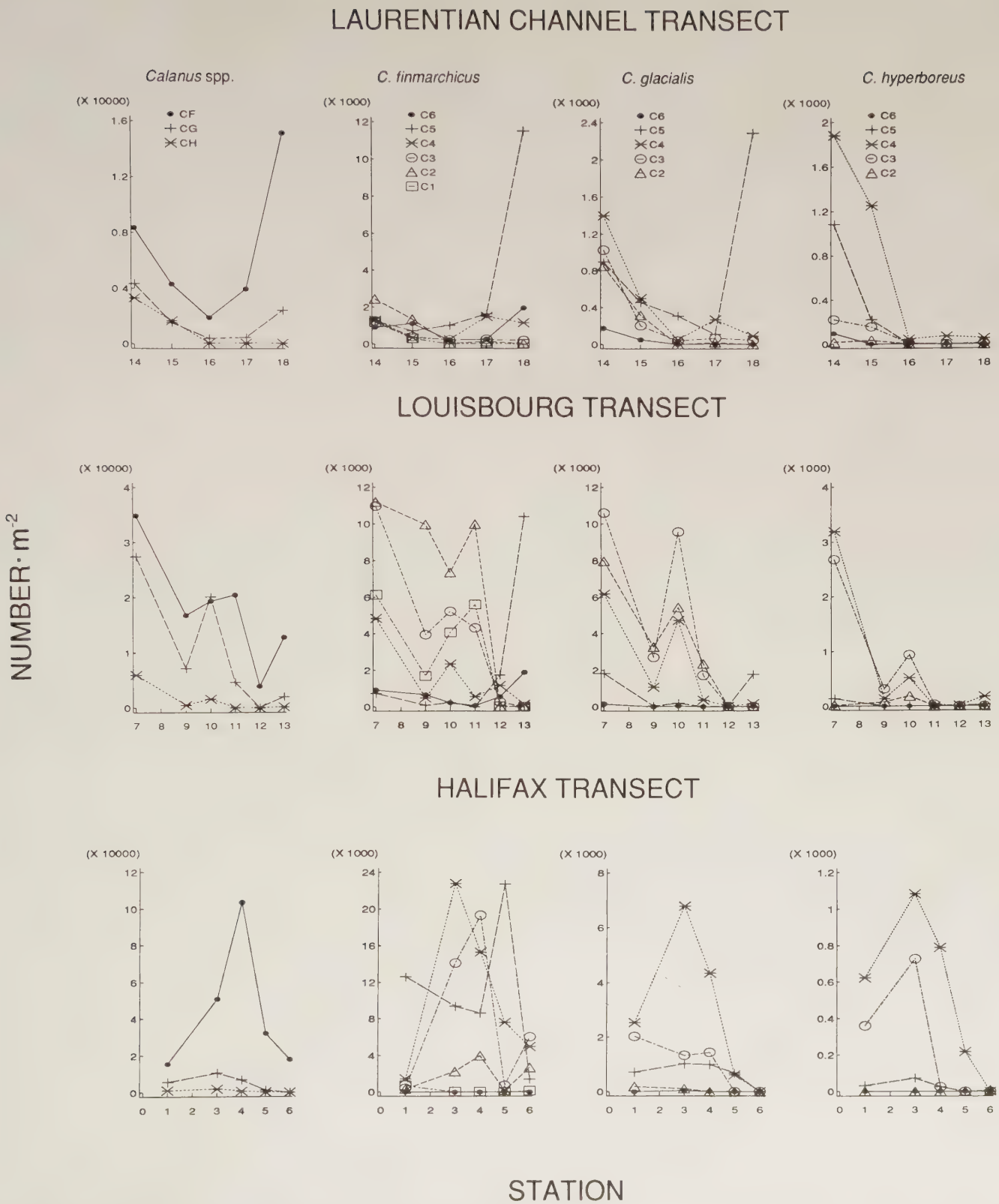

FIG. 7. Concentrations of total *C. finmarchicus* (CF), *C. glacialis* (CG), *C. hyperboreus* (CH), and various copepodite stages of each species during June 1985 on the different stations on the Laurentian Channel, Louisbourg, and Halifax transects. Note change of scale on the *y*-axis.

Shelf. Mechanisms by which freshwater runoff from the Gulf affects various fish stocks in the Gulf and along the Nova Scotia coast have been studied (Sutcliffe 1972, 1973; Sutcliffe et al. 1976; Bugden et al. 1982), and one mechanism suggested is increased phytoplankton production due to nutrient enhancement by the freshwater runoff. However, the introduction of copepods to the Shelf with the Gulf outflow has not been considered as a link between fish production and freshwater outflow from the Gulf. In light of this study, the possibility of such a linkage deserves attention.

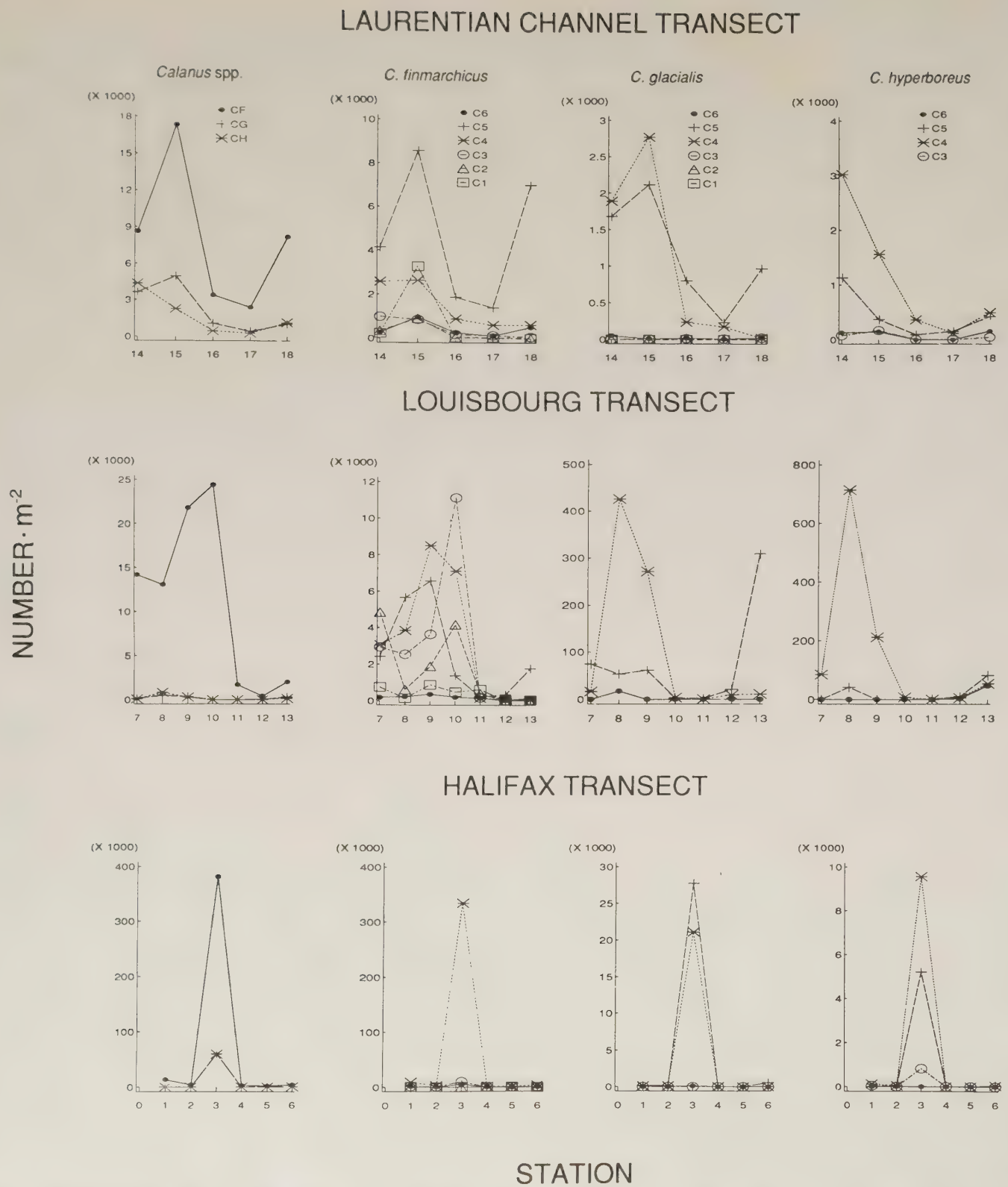

FIG. 8. Concentrations of total *C. finmarchicus* (CF), *C. glacialis* (CG), *C. hyperboreus* (CH), and various copepodite stages of each species during October 1985 on the different stations on the Laurentian Channel and Louisbourg transects. Note change of scale on the *y*-axis.

This study confirmed suggestions by Tremblay and Roff (1983) and Herman et al. (1991) that during the late winter and early spring the Gulf outflow introduced large numbers of early stages of *C. glacialis* and *C. hyperboreus* to the NE Scotian Shelf. It also demonstrated that *Temora* was carried onto the Shelf from the Gulf by the NSC. *Calanus finmarchicus* was probably also introduced to the Shelf by the NSC, as evidenced by the higher concentrations in zone 3 near the mouth of the Gulf. All three *Calanus* spp. from the NSC mixed with the resident populations that overwintered in the deep basins on the Shelf. These two sources made up the majority of *Calanus* populations on the Shelf. It is unlikely that populations of

C. glacialis and *C. hyperboreus* could exist long on the Shelf without the infusion of Gulf animals. The same may also be true for *C. finmarchicus*, but given the large populations of *C. finmarchicus* that overwinter in the basins, this species could probably maintain itself much longer than the other *Calanus* spp. without a significant input from the Gulf. What effect the size of the Gulf *Calanus* spp. infusion has on the overall Shelf production of these species is unknown. It is possible that during years of high river runoff and in turn greater Gulf outflow, the concentrations of *Calanus* spp. in the NSC are affected, thereby influencing the zooplankton production on the Shelf.

Acknowledgements

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Diel and Seasonal Changes in Resting Levels of Various Blood Parameters in Brook Trout[1] (*Salvelinus fontinalis*)

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Audet, C., and G. Claireaux. 1992. Diel and seasonal changes in resting levels of various blood parameters in brook trout (*Salvelinus fontinalis*). Can. J. Fish. Aquat. Sci. 49: 870–877.

Plasma osmolality, chloride, glucose, cortisol, thyroid hormones, and blood hematocrit all varied seasonally in freshwater-adapted, age 1+ brook trout (*Salvelinus fontinalis*) kept under natural photoperiod and water temperatures. In addition, plasma osmolality, cortisol, and thyroxine all displayed diel cycles that differed from month to month. High cortisol levels were related to sexual maturation and possibly to low water temperatures. Seasonal cycles of plasma thyroid hormones did not seem to be influenced by water temperature. Plasma osmolality, chloride, glucose, and thyroxine showed small but significant seasonal changes similar to the variations observed at the time of smoltification in other salmonid species.

Les niveaux plasmatiques d'osmolalité, de chlore, de glucose, de cortisol, d'hormones thyroïdiennes et l'hématocrite varient tous de façon saisonnière chez l'omble de fontaine (*Salvelinus fontinalis*) (1 an+) élevé en eau douce sous des conditions naturelles de photopériode et de température. De plus, l'osmolalité, le cortisol et la thyroxine plasmatiques varient de façon journalière, cycles journaliers qui diffèrent de mois en mois. De fortes concentrations de cortisol ont été observées en période de maturation sexuelle et pourraient être aussi reliées à la présence de basses températures. Les cycles saisonniers des concentrations plasmatiques des hormones thyroïdiennes ne semblent pas être reliés aux variations de température. Nous avons observé des variations saisonnières faibles mais significatives de l'osmolalité plasmatique de même que du chlore, du glucose et de la thyroxine, variations similaires à celles observées en période de smoltification chez d'autres espèces de salmonidés.

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Many populations of brook trout (*Salvelinus fontinalis*) are exclusively freshwater, while others undergo seasonal migrations into estuaries. In eastern Canada, brook trout move into salt water from most coastal streams. Although this species exhibits a great deal of variation in its movement patterns, the onset of seaward migration usually occurs in the spring (White 1941; Wilder 1952; Smith and Saunders 1958; Sutterlin et al. 1976; Dutil and Power 1980; Whoriskey et al. 1981; Castonguay et al. 1982). Brook trout do not show smoltification responses, and tolerance to seawater was reported to be size related (McCormick et al. 1985). McCormick and Naiman (1984a) did not find seasonal cycles in gill Na^+-K^+-ATPase activity in brook trout reared in freshwater, and they hypothesized that photoperiod affects seawater survival only through its influence on male sexual maturation (McCormick and Naiman 1984b). By contrast, Pelletier (1988) found seasonal differences in the ability of brook trout to tolerate direct transfer to seawater, these differences being related to different levels of gill Na^+-K^+-ATPase activity. Other experiments conducted under natural photoperiod, salinity, and temperature also showed seasonal differences in the hypoosmoregulatory ability of brook trout (G. Claireaux and C. Audet, unpubl. data). In that study, trout were able to acclimate to direct seawater transfer in spring or summer but not in fall and winter.

The purpose of the present work is to document whether or not seasonal and/or diel patterns in physiological parameters related to smoltification exist in brook trout, and further, if such patterns can be related to the osmoregulatory capability of these fish at certain times of the year.

Methods

Fish Holding Conditions and Experimental Design

Brook charr (age 1+) were obtained from a certified disease-free local hatchery and brought to the INRS aquaculture station in Pointe-au-Père, Québec (48°31′N, 68°29′W). They were held under natural temperature and photoperiod in a 3-m^3 circular tank provided with running dechlorinated tap water. Fish were acclimated to the laboratory rearing facilities for at least 1 mo before being used for experimental purposes. They were fed daily, usually in the morning, with commercial dry pellets (4 g·kg fish^{-1}), and feeding was discontinued 48 h prior to sampling. Annual and daily fluctuations in a variety of physiological and endocrine parameters were studied from nine sampling series of fish of both sexes randomly drawn from the stock tank. Sampling was done in August, September, October, and December 1988 (from eggs hatched in December 1986) and in February, March, April, May, and June 1989 (from eggs hatched in December 1987). On the 15th of each of these months, eight fish were sampled every 3 h over a 24-h period, i.e. at 10:00, 13:00, 16:00, 19:00, 22:00, 1:00, 4:00, and 7:00. Monthly average freshwater temperatures (±0.5°C) were as follows: February, 4°C; March, 4°C; April, 7°C; May, 9°C; June, 10°C; August, 14°C; September, 12°C; October, 10.5°C; December, 7°C. Average weight and length of trout sampled are presented in Table 1.

[1]The authors prefer the common name "charr" to designate species of the genus *Salvelinus*.

TABLE 1. Weight and length of brook trout sampled during the 24-h samplings. Results of the a posteriori tests of comparison of means are indicated by superscript letters a to e. Results are presented as mean ± SEM (n), n indicating the number of fish.

Month	Weight (g)	Length (cm)
February	60.9 ± 2.77^{a} (64)	18.4 ± 0.24^{a} (64)
March	69.7 ± 3.46^{ab} (64)	19.0 ± 0.29^{ab} (64)
April	72.8 ± 2.80^{ab} (64)	19.4 ± 0.24^{ab} (64)
May	81.2 ± 3.28^{bc} (64)	20.2 ± 0.27^{bc} (64)
June	89.8 ± 2.70^{c} (64)	20.7 ± 0.19^{c} (64)
August	139.8 ± 4.51^{d} (64)	23.5 ± 0.26^{d} (64)
September	134.8 ± 3.57^{d} (64)	23.8 ± 0.20^{d} (64)
October	169.8 ± 5.75^{e} (64)	25.1 ± 0.31^{e} (64)
December	173.5 ± 5.06^{e} (64)	25.9 ± 0.41^{e} (64)

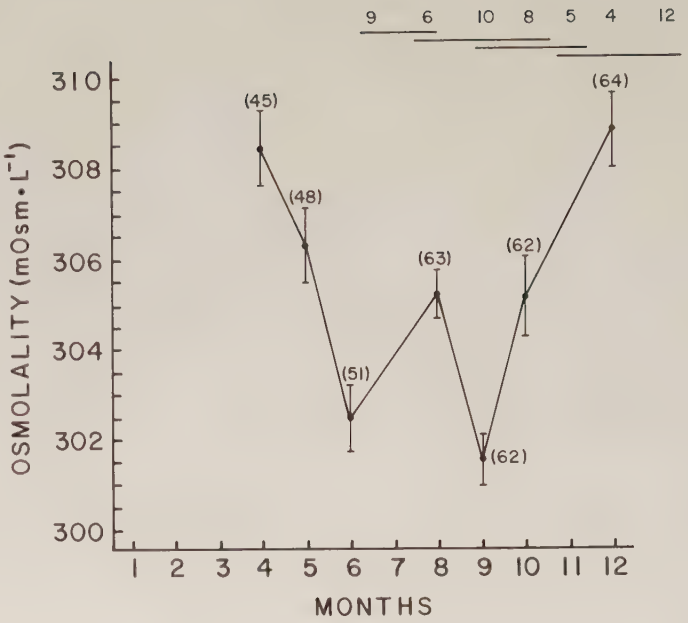

FIG. 1. Seasonal variations of plasma osmolality in brook trout reared in freshwater. The results of the a posteriori test are presented on the graph. The number of fish is indicated in parentheses. The numbers represent the different months and single lines underscore months between which there was no significant difference.

During the dark period, the rearing tank was isolated with black curtains; only minimal illumination was provided while netting fish to avoid injury to researchers. Fish were sampled one at a time. They were placed in a water bucket containing anaesthetic (0.02% MS 222 (3-aminobenzoic acid ethyl ester) neutralized with NaOH) and immediately taken away to an adjacent laboratory. After measuring body weight and length, blood was drawn by caudal puncture using ammonium heparinized syringes. From netting to caudal puncture, we proceeded as fast as possible and the average time lapse between these procedures was about 3 min. A small quantity of blood was kept for hematocrit and hemoglobin concentration measurements and the remaining blood was centrifuged at 7000g for 3 min. Plasma aliquots were then prepared, frozen on dry ice, and stored at −80°C for later analysis of plasma chloride, osmolality, glucose, cortisol, triiodothyronine (T_3), and thyroxine (T_4).

When smaller fish were sampled, the blood volume did not allow us to measure all the plasma parameters for each fish; therefore, hemoglobin data from February to April and plasma osmolality data for February and March are missing. These months had to be excluded from the statistical analysis because of the large number of missing values.

Hematocrit was determined after centrifugation in microcentrifuge tubes. Hemoglobin was measured by the cyanmethemoglobin method (Sigma procedure No. 525). Plasma chloride was measured with a Corning Chloride Analyzer 925 and plasma osmolality with an Advanced Micro-Osmometer 3MO. Plasma glucose was measured by an hexokinase enzymatic method (Sigma procedure No. 16UV). All measurements were done in duplicate and the average was used for statistical analysis. Cortisol, T_4, and T_3 were measured using a solid-phase (antibody-coated tube) technique (Kallestad procedure Nos. 825, 846, and 838, respectively). Large brook trout were sampled and their plasma separated into aliquots for further determination of intra- and interassay coefficients of variation and to prepare serial dilutions of fish plasma for specificity checking. For each of the three radioimmunological assays, serial dilutions of fish plasma were assayed and the slope of the displacement curves compared with the slope of the standard curves. For cortisol, T_3, and T_4 assays, these slopes were found to be homogeneous (ANCOVA, $\alpha = 0.001$). Large brook trout were killed and the plasma was separated into aliquots for determination of intra- and interassay coefficients of variation. For T_3, they were 4.8 and 5.8%, respectively. Preliminary measurements showed that plasma cortisol and T_4 concentrations in brook trout were often below the average human plasma concentrations for which the kits had been designed. Dilutions of the standards were then prepared using the free hormone human serum in order to increase the range of the standard curve. With brook trout plasma, the intra- and interassay coefficients of variation were, respectively, 8.7 and 8.3% for cortisol and 10.3 and 3.4% for T_4. When plasma concentrations were too low to be adequately measured, they were assigned the detection limit of the modified assay (0.03 $\mu g \cdot 100\ mL^{-1}$ for cortisol and 0.07 $\mu g \cdot 100\ mL^{-1}$ for T_4). All measurements were done in duplicate; when the deviation from the mean was more than 5%, a third dosage was done. The average of the difference between duplicates expressed as a percentage of the mean values was 0.97% for cortisol, 1.73% for T_3, and 0.62% for T_4 measurements. Averages were used for statistical analysis. Calculation of radioimmunoassay results was made with a LKB Clinigamma 3 counter utilizing the spline-function program.

Statistics

Except for cortisol and T_4, data have been routinely expressed as mean ± SEM(n) where n represents the number of fish. Normality and homogeneity of variances were checked by Kolmogorov–Smirnov and F_{max} tests, respectively. Some sets of data had to be transformed prior to statistical analysis in order to obtain homogeneity of variances: blood hemoglobin and plasma T_3 concentrations were transformed as log ($x + 1$) and

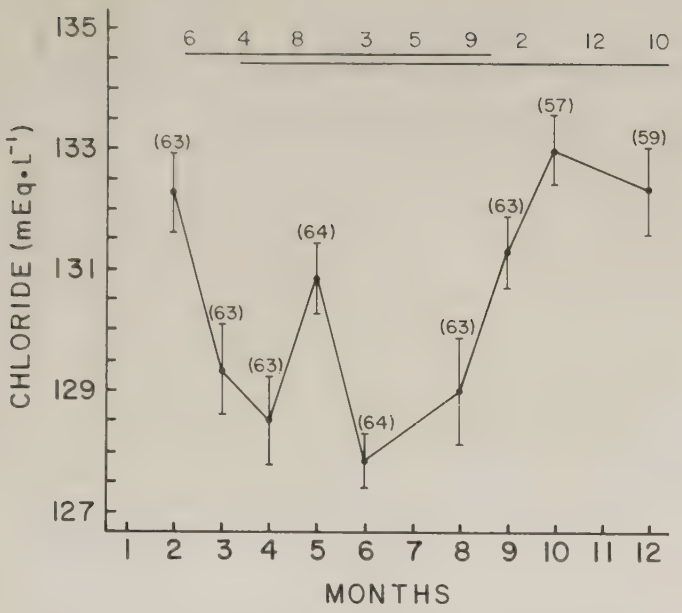

FIG. 2. Seasonal variations of plasma chloride in brook trout reared in freshwater. See Fig. 1 for other details.

plasma glucose as $1/x$. The data were analyzed by two-way ANOVA ($\alpha = 0.05$); time of sampling (hour) and period of the year (month) were used as sources of variation. A posteriori T' tests of comparison of means (Sokal and Rohlf 1981) with $\alpha = 0.05$ were applied following ANOVAs. For those parameters for which transformations failed to give homogeneity of variances, we applied the Games and Howell test for comparison of means, designed for heterogeneous variances (Sokal and Rohlf 1981). When the two-way ANOVA indicated significant interactions between the two sources of variation (hour, month) for a given parameter, each transfer was a posteriori analyzed for this parameter by one-way ANOVAs (hour) in order to identify the nature of the seasonal differences in the course of the daily cycles.

Plasma cortisol and T_4 data were not normally distributed and have been presented using the multiple box-and-whisker plot. The data were analyzed by Kruskall–Wallis ANOVAs. A first analysis was performed on the overall data set to check for seasonal differences in average plasma hormone levels and to look for general diel effects. For each month, a further analysis was performed to look for the presence or absence of diel cycles. When significant effects were found, the Kruskall–Wallis test was followed by an a posteriori LSD test on ranks (Daniel 1978).

Results

Both plasma osmolality (Fig. 1) and chloride (Fig. 2) showed significant seasonal variations in fish reared in freshwater ($p \leq 0.001$). Plasma osmolality and chloride both decreased gradually from winter to summer and increased again in late fall. For these two parameters, values measured in June were significantly lower than the ones measured in December and February. As already mentioned, osmolality data for February and March were not included in the analysis and are not presented in the figures. Nevertheless, their respective means were 315.7 ± 0.99 (30) and 311.4 ± 1.48 (34). They were

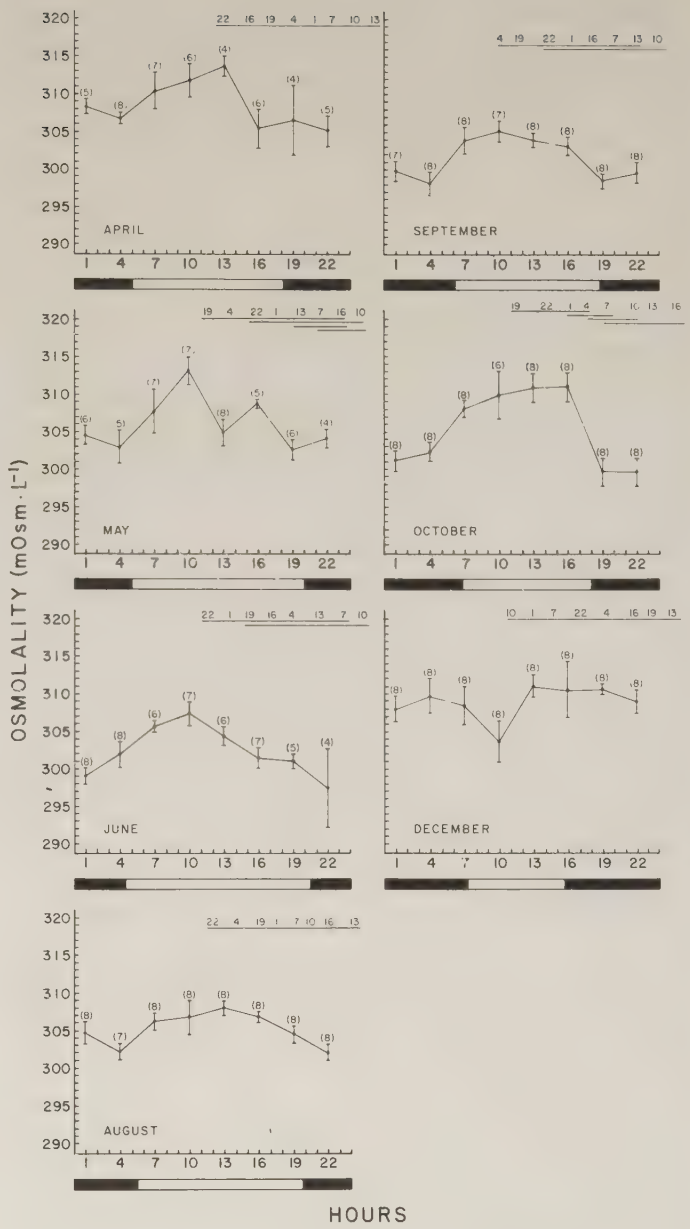

FIG. 3. Seasonal changes in diel variations of plasma osmolality in brook trout reared in freshwater. Photoperiod conditions are illustrated below each graph. See Fig. 1 for other details.

homogeneous with the December measurements but significantly higher than those in June when tested by one-way ANOVA and an a posteriori test on months. The very low plasma osmolality level observed in September was transient and was not correlated with any change in plasma chloride concentration.

Only plasma osmolality showed significant diel variations ($p \leq 0.001$) (Fig. 3). For this parameter, significant interactions between both sources of variation (hour, month) were also found ($p \leq 0.001$). Overall diel variations in plasma osmolality were characterized by a clear difference between light and dark periods, with osmolality being higher during the hours of illumination. In Fig. 3, we can see that sunrise was usually accompanied by a rise in plasma osmolality that peaked around midday, followed by a decrease that was often complete by sunset. For each month showing a significant diel cycle, the

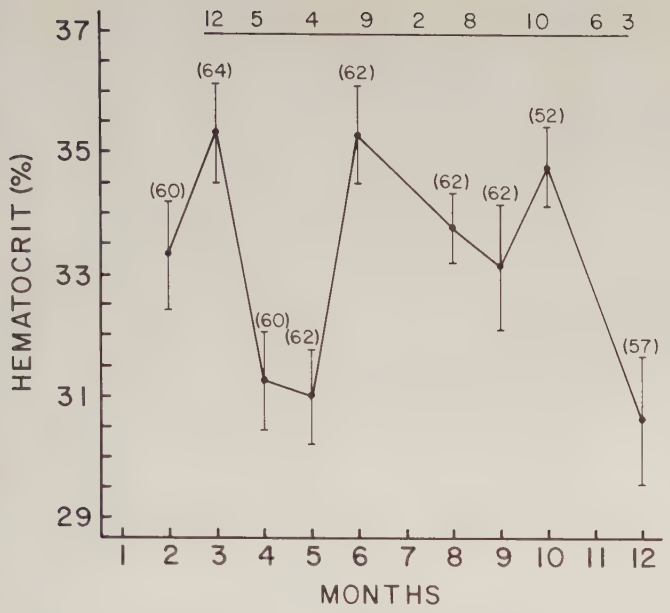

FIG. 4. Seasonal variations of blood hematocrit in brook trout reared in freshwater. See Fig. 1 for other details.

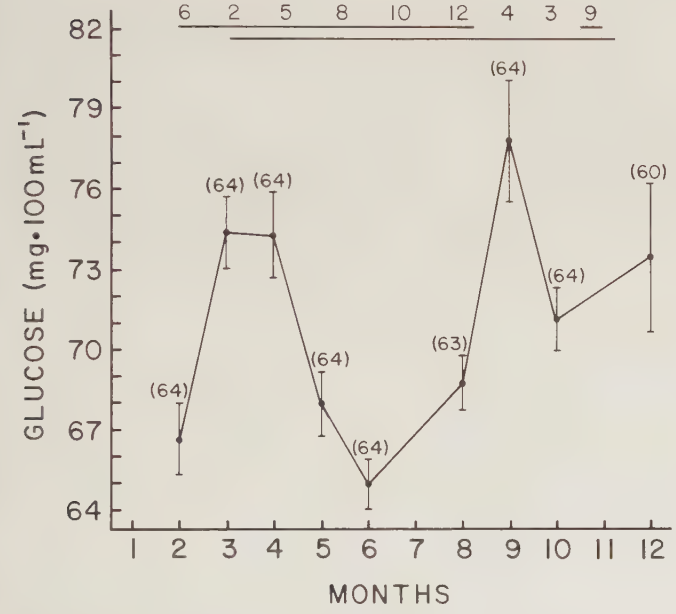

FIG. 5. Seasonal variations of plasma glucose in brook trout reared in freshwater. See Fig. 1 for other details.

highest plasma osmolality was always observed at 10:00, while lowest osmolality was generally observed between 19:00 and 22:00.

ANOVA demonstrated a significant seasonal effect on blood hematocrit ($p \leq 0.001$), but no significant daily variations were found ($p > 0.05$) (Fig. 4). The a posteriori analysis ($\alpha = 0.05$) failed to show any significant differences among months, indicating that the seasonal changes were not pronounced. Neither season nor time of day significantly influenced the blood hemoglobin concentration ($p > 0.05$), the overall mean in freshwater brook trout being 7.16 ± 0.08 (317) g·100 mL^{-1}.

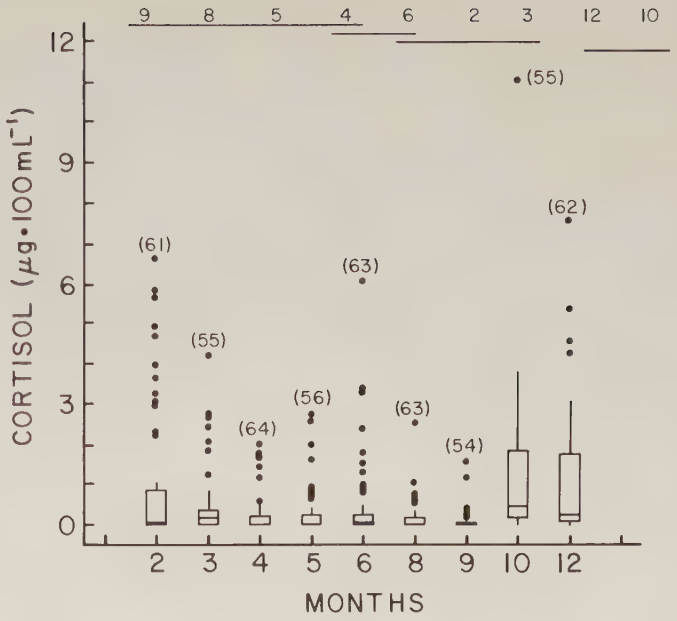

FIG. 6. Seasonal variations of plasma cortisol in brook trout reared in freshwater. Each box covers the middle 50% of the data values (between the lower and upper quartiles) and the whiskers extend out to the minimum and maximum values, while the central line is at the median. The points beyond 1.5 times the interquartile range are plotted as individual adjacent values. See Fig. 1 for other details.

Significant seasonal but not diel effects on plasma glucose were found (factor month, $p \leq 0.001$; factor hour, $p > 0.05$; interactions, $p > 0.05$). As observed for osmolality and chloride, low plasma glucose concentration was observed in June (Fig. 5). This concentration was significantly lower than concentrations observed in early spring and September.

Both season and time of day had a significant effect on plasma cortisol concentration ($p \leq 0.001$). The seasonal analysis showed that plasma cortisol decreased from February to September and then increased again (Fig. 6). In terms of diel variations, the lowest cortisol values were generally observed during the middle of the day (Fig. 7). In February, plasma cortisol levels were highest at sunrise and sunset, remaining relatively high during the dark period. Low levels were observed during the light period. In March, October, and December, no significant diel variations were observed ($p > 0.05$). In April, May, and June, the highest plasma cortisol concentrations were observed during the dark period with low average levels found during the light period. The same trend was seen in September, although smaller differences in measurements were noted.

Plasma T_4 displayed both significant seasonal and daily variations ($p \leq 0.01$). A gradual decrease was observed from February to May followed by an increase to a maximum in June (Fig. 8). Levels then decreased until September and rose again in late fall and winter. Diel variations of T_4 were present for all months except September and February (Fig. 9). During spring and summer, diel variations of T_4 were characterized by the presence of high concentrations during the day. In September, plasma T_4 remained low during the whole 24-h period. In fall and winter, an additional rise occurred during the night.

Seasonal variations in plasma T_3 were observed ($p \leq 0.001$), but no diel cycles were present ($p > 0.05$). From February to May, plasma T_3 concentration decreased by 57% (Fig. 10). A major increase then occurred in August, followed by an immediate return to values similar to those observed in May.

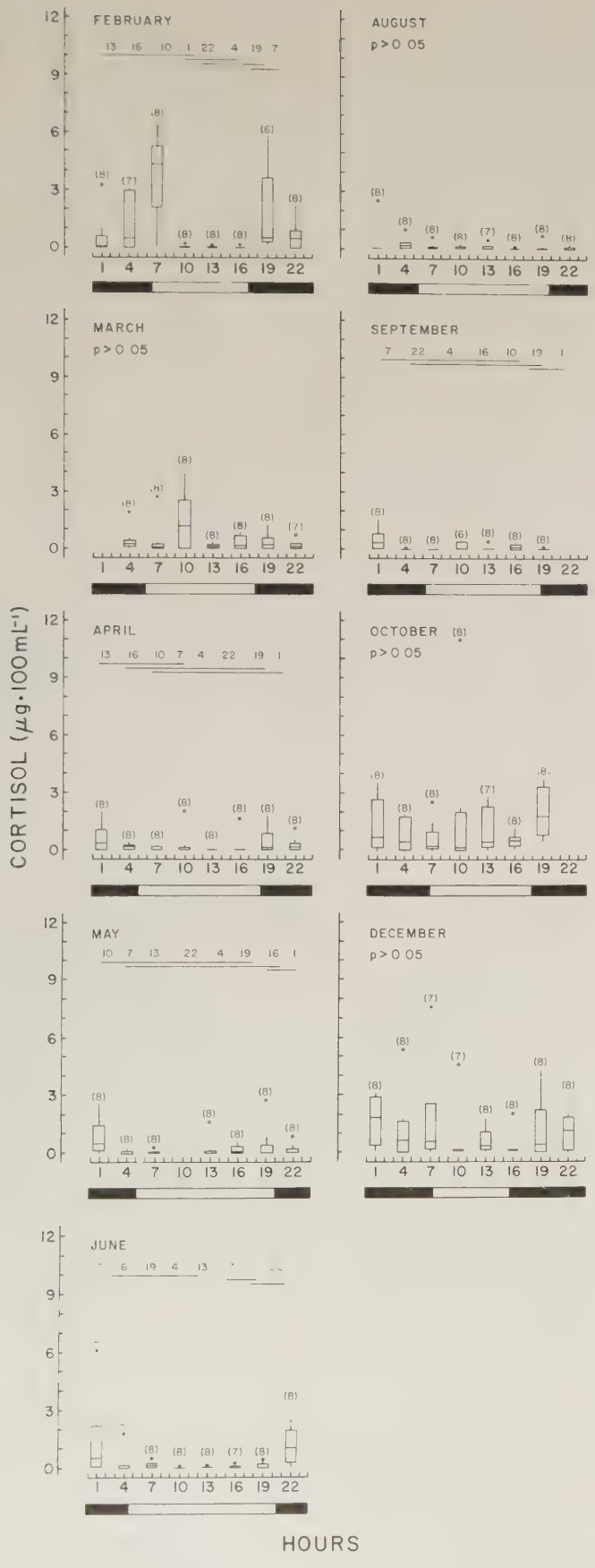

FIG. 7. Seasonal changes in diel variations of plasma cortisol in brook trout reared in freshwater. See Fig. 1 and 6 for other details.

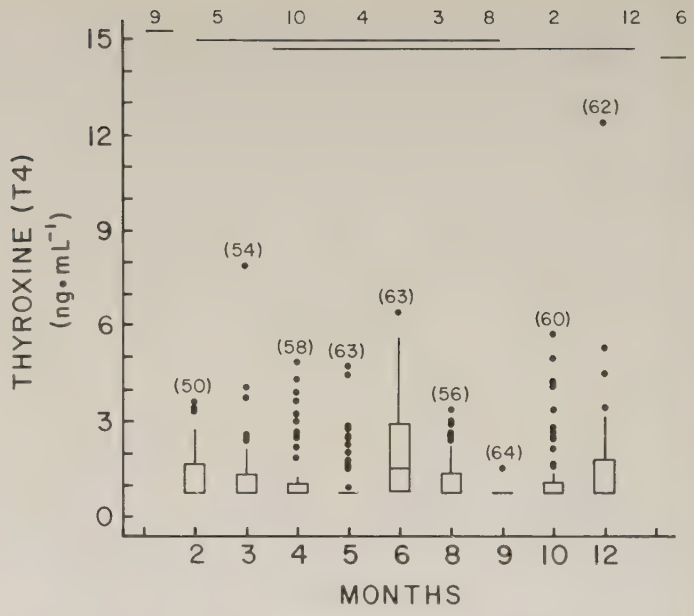

FIG. 8. Seasonal variations of plasma T_4 in brook trout reared in freshwater. See Fig. 1 and 6 for other details.

Discussion

This study points out that seasonal and diel patterns exist in several physiological parameters in brook trout.

Diel variations in plasma osmolality were not accompanied by diel variations in plasma chloride. On the other hand, the seasonal variations in osmolality and plasma chloride were quite similar. They were characterized by higher levels in late fall and winter and generally lower levels in spring and summer. Even when they were significant, the intensity of these variations was low. Plasma osmolality, chloride, and glucose were all at their lowest level in June. These lower plasma concentrations could be the consequence of an increase in plasma water content. Indeed, a drop in plasma osmolality observed in salmonids at the time of smoltification and seawater migration has been associated with a transient loss of hyperosmoregulatory ability (e.g. Thorpe 1988; Birt et al. 1990). However, the fact that the lowest annual muscle water content in freshwater fish from this stock was also observed in June (G. Claireaux and C. Audet, unpubl. data) is contradictory.

In salmonids, hematocrit, hemoglobin, and plasma glucose concentration are influenced by seasonal water temperatures (e.g. Cunjak and Power 1986; Virtanen 1987). In this study, we found a significant overall seasonal effect on hematocrit, although we were unable to discriminate between the different months of sampling; no seasonal differences were found for hemoglobin. McCormick and Naiman (1984a) demonstrated sex-related seasonal differences in hematocrit, a relationship not observed in the present study. Glucose also did not seem to have been influenced by temperature, as no correlation could be detected between changes in freshwater temperature and plasma glucose concentration.

A significant seasonal cycle in plasma cortisol levels was also observed, with higher levels in winter and lower levels in spring and summer. In contrast with other months, plasma cortisol levels were consistently high in October but with no detectable diel cycle. This could possibly have been related to the period of sexual maturation, as maximum gonadosomatic index

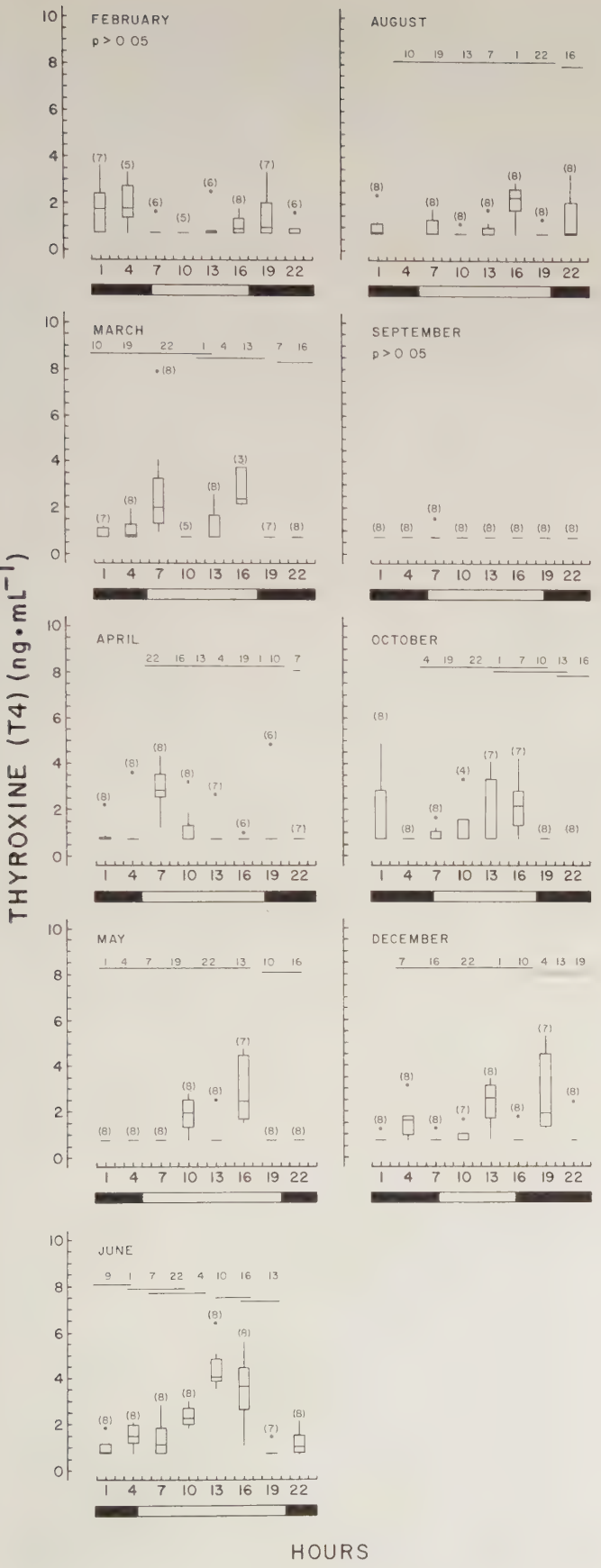

FIG. 9. Seasonal changes in diel variations of plasma T_4 in brook trout reared in freshwater. See Fig. 1 and 6 for other details.

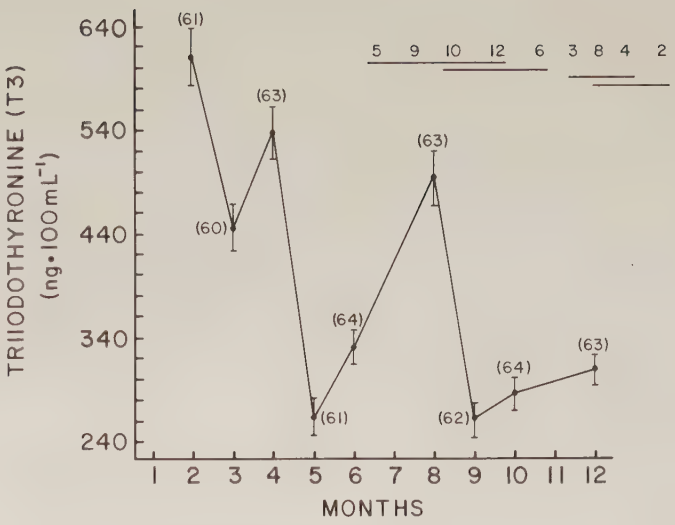

FIG. 10. Seasonal variations of plasma T_3 in brook trout reared in freshwater. See Fig. 1 for other details.

in our fish stock was observed in October. Pickering and Christie (1981) showed that sexual maturation in brown trout (*Salmo trutta*) was characterized by a marked elevation in plasma cortisol levels. The presence of high plasma cortisol levels during winter could have been related to low temperatures. In contrast with smoltifying salmonids, where spring cortisol peaks have sometimes been observed (Virtanen and Soivio 1985; Langhorne and Simpson 1986; Soivio et al. 1989), cortisol in the present study was higher at the time of year when trout transferred to seawater failed to acclimate (fall and winter) (G. Claireaux and C. Audet, unpubl. data). Most of the other months were characterized by the presence of diel cycles, with maximum cortisol values observed during the dark period. Such results correspond to a certain extent with observations made on brown trout and Atlantic salmon (*Salmo salar*) (Pickering and Pottinger 1983; Nichols and Weisbart 1984; Thorpe et al. 1987). In contrast with our October observations, we found low levels of cortisol in August with no detectable diel cycle.

Both thyroid hormones (T_3 and T_4) showed strong seasonal variations. This confirms the findings of White and Henderson (1977), who showed maximum values for both T_3 and T_4 in midspring and minimum values at the time of spawning (late October, beginning of November for that string) in brook trout. In addition, the range of circulating hormone levels is similar in both studies. In our study, both cycles were not as well synchronized as those observed by White and Henderson: plasma levels of both hormones were low in September and October, while plasma T_3 reached a peak earlier in the spring than T_4. The overall seasonal changes in plasma T_4 levels that we found are also only slightly different from those observed by McCormick and Naiman (1984a) under natural photoperiod but constant water temperatures, i.e. high spring levels, low summer levels, and a secondary peak in autumn. Despite differences in temperature regimes (10°C, McCormick and Naiman 1984a; 0–5°C, White and Henderson 1977; 4–15°C, this study), the results of the three studies are similar. This suggests that seasonal variations in water temperature may not play an essential role in the establishment of seasonal cycles of thyroid hormones. Nevertheless, temperature has been shown to have important effects on the metabolism of thyroid hormones in brook trout (Drury and Eales 1968; Vijayan et al. 1988). These

studies indicated that the metabolism of T_4 and its conversion to T_3 increased as water temperatures increased. When our T_3/T_4 ratios were ranked from lowest to highest, the results were as follows: June, 0.62; May, 0.86; December, 0.91; October, 0.93; September, 1.11; March, 1.40; August, 1.46; April, 1.62; and February, 1.78. If conversion of T_4 to T_3 also increased with rising temperatures in the present study, it implies that T_3 metabolism would also have been considerably activated with rising temperature. However, these ratios considered alone would instead indicate a decreased conversion of T_4 to T_3 with rising water temperature. We cannot speculate on what really happened in the fish we studied because periodic plasma measurements are not representative of what is happening in terms of rate of secretion, deiodination, or degradation of these hormones.

Low levels of thyroid hormones during the fall may be related to spawning time (White and Henderson 1977; Sower and Schreck 1983). High plasma T_4 concentration peaks are usually associated with smoltification and are often correlated with increased Na^+-K^+-ATPase activity. We did not measure the Na^+-K^+-ATPase activity, but a study carried out by Pelletier (1988) under similar experimental conditions (i.e. following natural photoperiod and water temperature regime) showed a significant increase in Na^+-K^+-ATPase activity in brook trout held in freshwater in late spring (May and June) compared with summer and fall. In the present study, the highest monthly plasma T_4 concentration was observed in June. McCormick et al. (1985) did not observe significant changes in T_4 between May and August in a nonanadromous brook trout population, but they did find significantly higher T_4 levels in an anadromous population migrating downstream in June. Interestingly, plasma T_4 concentrations found in the present study in a fish-farmed stock were similar to the levels found by McCormick et al. (1985) in the anadromous population.

Diel variations of T_4 were observed along with seasonal variations. In spring and summer, high plasma T_4 concentrations were observed only during the light period, while in fall and winter, elevated plasma T_4 concentrations were also observed during the dark period. September was unusual in that there were low levels of plasma T_4 throughout the 24-h period. High levels of T_4 during the day in spring and summer were also reported by McCormick and Naiman (1984a). The role of these diel cycles has yet to be explained, but the phase shift from month to month is likely of some biological importance. The presence of maximum concentrations during the light period in spring and summer and during the dark period from October to February is especially interesting. Photoperiod is one important factor, but temperature conditions, and perhaps even evolution of temperature conditions throughout the life history of the fish, are certainly as important. It is generally postulated that in regulation of the numerous changes associated with downstream migrations, photoperiod would entrain the many endogenously controlled developmental rhythms, while temperature would act to govern the rate of physiological responses (Thorpe 1988). The knowledge of natural endogenous rhythms and the effects of environmental factors on these rhythms as well as on the response to seawater transfer is a prerequisite to our attempts to modulate seawater adaptability in brook trout for marine aquaculture purposes.

Acknowledgements

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Progression of Coronary Arterial Lesions in Atlantic Salmon (*Salmo salar*) as a Function of Growth Rate

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Saunders, R. L., A. P. Farrell, and D. E. Knox. 1992. Progression of coronary arterial lesions in Atlantic salmon (*Salmo salar*) as a function of growth rate. Can. J. Fish. Aquat. Sci. 49: 878–884.

Development of coronary arteriosclerosis has been studied in all life stages of both wild and cultured Atlantic salmon (*Salmo salar*). Lesions accumulate slowly during juvenile growth in freshwater in both wild and cultured populations and increase sharply during early marine life; much of the progression occurs well before the onset of sexual maturity. Fewer than 5% of sexually mature Atlantic salmon (wild or fast-growing cultivated) were free of coronary lesions. Lesions were distributed over 45–85% of the length of the coronary artery. Lesions were less abundant and less advanced in a cultured strain of salmon that grew more slowly than another cultured, fast-growing strain. We conclude that prevalence and severity of lesions in Atlantic salmon accelerate in parallel with, and possibly in response to, those factors responsible for rapid growth in the sea. We discuss diet, endocrine changes associated with sexual maturation, metabolic aspects of parr–smolt transformation, and physiological stress as factors possibly influencing progression of lesions associated with rapid growth during the marine stage.

On a étudié l'évolution de l'artériosclérose coronarienne chez tous les stades du cycle vital du saumon de l'Atlantique (*Salmo salar*) sauvage et d'élevage. Le nombre de lésions a augmenté lentement au cours de la période de croissance des juvéniles sauvages et d'élevage vivant en eau douce, puis a augmenté brusquement au début du séjour en eau salée. Cette augmentation a eu lieu en grande partie bien avant le début de la maturité sexuelle. Moins de 5 % des saumons sexuellement matures (sauvages ou d'élevage à croissance rapide) étaient libres de lésions coronaires. De 45 % à 85 % de la longueur de l'artère coronaire portait des lésions. Celles-ci étaient moins abondantes et moins élaborées chez une souche de saumon d'élevage à croissance plus lente pa rapport à autre souche d'élevage à croissance rapide. Les auteurs formulent la conclusion que la fréquence et la gravité des lésions chez le saumon de l'Atlantique progressent rapidement, en parallèle et peut-être en réaction aux facteurs responsables de la croissance rapide en mer. Ils examinent le régime alimentaire, les réactions des fonction endocrines liées à la maturation sexuelle, certains aspects métaboliques de la transformation des tacons en saumoneaux et le stress physiologique à titre de facteurs qui pourraient influer sur l'évolution des lésions associées à la croissance rapide au cours du séjour en eau salée.

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Coronary arteriosclerosis in salmonids, first reported over 25 yr ago (Robertson et al. 1961), typically involves myointimal hyperplasia (Van Citters and Watson 1968; Maneche et al. 1972; Moore et al. 1976). Smooth muscle cells proliferate within the vessel lumen, resembling the early stages of mammalian coronary arteriosclerosis (House and Benditt 1981), resulting in lesions that are initially small and focal. These lesions may merge and increase in size; they commonly increase in severity and can narrow the lumen significantly, particularly in sexually maturing salmonids (Farrell et al. 1986, 1990). The internal elastic lamina is disrupted but lipid and calcium deposits are not generally found in association with these lesions (Robertson et al. 1961; Van Citters and Watson 1968; Maneche et al. 1972; Moore et al. 1976; McKenzie et al. 1978; Eaton et al. 1984). Moderate-to-severe lesions are generally found in salmonids approaching spawning condition (Robertson et al. 1961; Van Citters and Watson 1968; Moore et al. 1976; McKenzie et al. 1978; Eaton et al. 1984). This is a time when salmon place great demands on their cardiovascular systems, migrating upstream, avoiding predators, and fighting rival spawners. Although the salmonid coronary circulation supplies only the epicardial portion of the heart, adequate coronary blood flow is essential for maximum aerobic swimming (Farrell and Steffensen 1987). Discovery of the primary factor or factors associated with lesion development is of interest, especially in view of the high levels of ω-3 fatty acids normally found in salmonids (Phillipson et al. 1985; Ackman et al. 1988). While humans have increased their dietary intake of fish and fish oils in an effort to curb cardiovascular disease and atherosclerosis, salmonids are apparently immune to the protective effect of ω-3 fatty acids insofar as proliferation of vascular smooth muscle in the intima of the coronary artery is concerned.

Since most observations of coronary arterial lesions in salmonids have been in sexually mature individuals, sexual maturation has been suggested as the primary factor associated with arteriosclerosis. Precocious sexual development promotes lesion formation (House et al. 1979), and severe lesions are typically observed in sexually mature salmonids (Farrell et al. 1986; Kubasch and Rourke 1990). Injection of sex hormones increased the severity of lesions in juvenile steelhead trout (*Oncorhynchus mykiss*) (Schmidt and House 1979). However, it is more likely that sexual maturation is, at most, an important secondary factor, since immature Atlantic salmon (*Salmo salar*), either in cultivation or caught in high-seas fisheries,

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TABLE 1. Numbers and size range (fork length) of wild and cultured Atlantic salmon sampled at various life stages.

Life stage	Size range (cm)	Wild	Cultured
Fry and parr	5–13	34	99
Smolt	14–18	255	97
Postsmolt	19–35	44	159
Marine feeding, mature, spawning, or kelts	>36	184	553
Total		517	908

usually have a high incidence of lesions at least 1 yr prior to sexual maturation (Farrell et al. 1986). Moreover, lesions continued to develop in Atlantic salmon after they had spawned (Saunders and Farrell 1988) and, in fact, further lesion development closely paralleled the resumed growth. The primary factor associated with lesion development is therefore unclear, but lesions clearly have their beginning in immature Atlantic salmon.

We have conducted a comprehensive study of the prevalence and severity of coronary lesions in all life stages of Atlantic salmon. Our objective was to gain insight into the factor or factors affecting lesion development and to test the hypothesis that coronary arterial lesions in salmonids are promoted primarily by growth or factors associated with growth. Accordingly, we sampled all life stages of wild and cultured Atlantic salmon, with samples of each stage collected at one or more geographic locations.

Materials and Methods

Wild Atlantic salmon (n = 517), which normally spawn at ages 4–7 yr, were sampled at various life stages from a number of freshwater and marine areas in eastern Canada and in the sea near Greenland. Cultured salmon (n = 908), which mature under accelerated growth conditions in about 30 mo, were sampled as juveniles at the St. Andrews Biological Station and during their marine stages at a number of marine salmon farms in southwestern Bay of Fundy, New Brunswick. Table 1 shows the number and approximate size range of individuals sampled at the freshwater and marine stages. Scales for use in determination of age were not available for most fish collected. Sex of sampled fish was not always recorded. Therefore, no attempt was made to determine the age of wild salmon which, at a given developmental stage, were older than comparable cultured stages. In any case, wild salmon are of different ages at given developmental stages owing to environmental variation among nursery rivers and marine feeding locations, as well as to growth and developmental differences among genetically distinct stocks (Saunders 1981). Therefore, age and sex were not taken into account in the principal analysis that compared lesion prevalence and severity between wild and cultured salmon in relation to size.

Comparisons of lesion development in fast- and slow-growing salmon were based on cultured strains with markedly different marine growth rates. Cultivated strains developed from the rapidly growing Saint John River, New Brunswick, stock and the slower growing Western Arm Brook, Newfoundland, stock are described in Chadwick et al. (1978) and Saunders et al. (1987). Juveniles were reared to the smolt stage at the St. Andrews Biological Station. Growth to the 1-yr smolt stage was comparable between groups. Smolts of about 18–20 cm length were acclimated to seawater and moved to a marine sea cage where growth was monitored periodically during 16 mo, after which a portion of the fish matured sexually (Saunders et al. 1987). Data from these Saint John and Western Arm Brook fish were used to compare lesion prevalence and severity between rapidly and slowly growing strains reared separately but at the same time in the freshwater and marine facilities described above. The data were also used, together with those from other cultured salmon, for comparison between cultured and wild salmon. Samples of cultured and wild salmon were grouped into 5-cm length classes for a detailed analysis of size-related lesion prevalence and severity.

The heart, ventricle, and bulbus arteriosus were removed from freshly killed fish and placed in a 10% buffered formalin solution, stored, and examined histologically as described by Farrell et al. (1986) and Saunders and Farrell (1988). The hearts were dehydrated, cleared, and embedded in paraffin wax (Tissue prep.) for sectioning at 5 μm thickness on an 820 AO rotary microtome. The coronary artery runs caudad along the ventral surface of the central aorta and bulbus arteriosus. Most sections of the coronary were taken cephalad of the first bifurcation of the artery. Sections were stained with Verhoff's elastic stain and counterstained with picroponceau. About 10 sets of serial sections were taken from five or more sites along the coronary artery and a minimum of 50 (as many as 150) arterial cross sections were graded for prevalence and severity of lesions per fish.

An artery without lesions was assigned a severity index of 0; lesion severity was ranked from 1 to 4 based on the system of Moore et al. (1976). Grades 1–3 represent a progressive increase in lesion severity, indicative of the number of smooth muscle cells found within the lumen of the artery; grade 4 was assigned to severe lesions. Lesion severity index (LSI) of each fish was determined by the formula

$$(1 \times n_1) + (2 \times n_2) + (3 \times n_3) + (4 \times n_4)/(n_1 + n_2 + n_3 + n_4)$$

where n = the number of cross sections with a grade equivalent to the subscript. Percent prevalence of lesions was calculated for each fish from 100 × the number of cross sections with a grade greater than 0 ÷ the total number of cross sections graded. Since cross sections were taken at several random locations along the length of the artery, lesion prevalence for a given fish is a reasonable estimate of the distribution of lesions along the length of the main coronary artery.

The data were analyzed by one-way ANOVA; the lesion prevalence data were analyzed after arcsine transformation.

Results

Both wild and cultured Atlantic salmon have low prevalence of mild lesions at early stages corresponding with juvenile life in freshwater (Fig. 1). Although lesions were not found in fry, they became progressively more prevalent and more severe as the fish grew longer. Lesions were progressively more prevalent and severe in salmon during their marine phase. The lesions are larger and more numerous in rapidly growing and maturing marine salmon (Fig. 2). The extent of lesions ranges from a small collection of smooth muscle cells to a large mass which occludes up to 50% of the cross-sectional area of the artery (Fig. 3). Among mature fish, fewer than 5% of the sample population were free of lesions, and lesions were distributed over 45–85% of the length of the coronary artery.

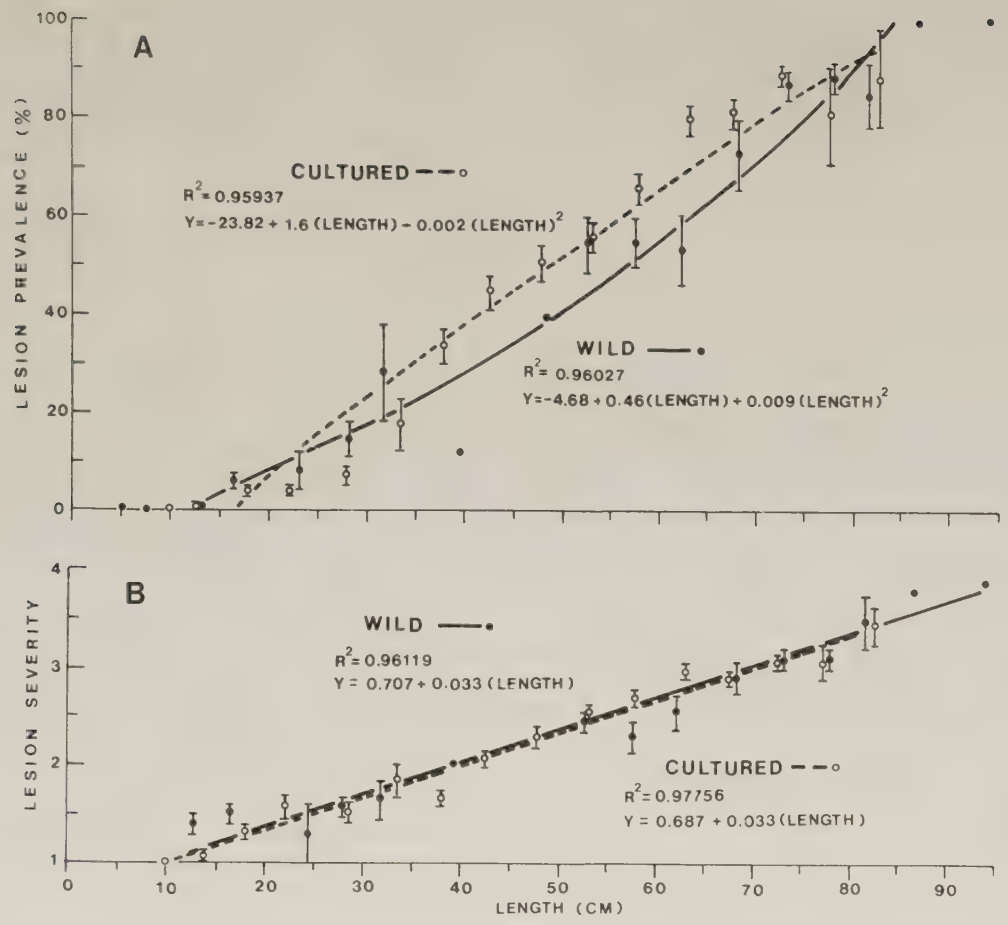

FIG. 1. (A) Prevalence and (B) severity of arteriosclerotic lesions in coronary arteries of wild (n = 517) and cultured (n = 908) Atlantic salmon at various life stages. Each point represents a mean value (bar = SEM) for various numbers (4–97 cultured, 3–190 wild) of fish grouped into 5-cm length classes. Points are plotted at mean lengths within length classes. Points without SEM represent single fish.

There is a clear, direct relationship between fish length and prevalence of coronary lesions in both wild and cultured Atlantic salmon sampled at various life stages from juveniles in freshwater to smolts and postsmolts beginning their marine life and the later marine feeding stages leading to return migration, spawning, and postspawning (kelt) recovery (Fig. 1). There is a similar relationship between fish length and the LSI (Fig. 1).

Second-degree polynomial equations (Fig. 1) adequately describe the correlation between bodily growth and prevalence and severity of lesions ($R^2 > 0.95$ for all relationships). The quadratic term was not statistically significant. Thus, the relationships between fish length and both prevalence and severity of lesions were not significantly different between wild and cultured salmon. The wild salmon in our sample were older, at given developmental stages and sizes, than their cultured counterparts. Cultured salmon may reach the smolt (migratory) stage at 1 yr; wild smolts in eastern Canada are from 2 to 5 yr old, depending on riverine growing conditions. Cultured salmon reach sexual maturity in as little as 30 mo from first feeding; wild salmon may reach maturity at 42 mo, but most take 54 or 66 mo. Although wide variability precludes meaningful statistical evaluation of these size classes with respect to lesion prevalence, it is obvious that cultured salmon attain larger size in a shorter time and have equal or greater prevalence and severity of lesions compared with wild salmon at given life stages. Thus, since the prevalence of lesions is highly correlated with size (Fig. 1), it can be safely deduced that accelerated growth rate, or some factor associated with growth, is a key factor contributing to progression of coronary lesions.

The lack of age data for the sample of wild salmon precluded a detailed analysis of whether or not faster growth rate of cultured salmon affected lesion progression directly. To confirm that lesion development was influenced by growth rate, we took advantage of the opportunity to examine growth rate and lesion progression in two cultured strains which grew at different rates. The fast-growing Saint John River (New Brunswick) and slow-growing Western Arm Brook (Newfoundland) strains grew at rates comparable to the smolt stage in freshwater but at different rates in sea cages. Growth and progression of lesions was assessed for 16 mo of marine life, by which time approximately 10 and 60% of the New Brunswick and Newfoundland fish, respectively, had become sexually mature. Again, there were significant correlations between fish length and both lesion prevalence (lesion prevalence = 9.71 − 1.0(length) + 0.035(length)^2; R^2 = 0.95) and lesion severity (lesion severity = 0.656 + 0.03(length); R^2 = 0.98) for the Newfoundland strain. However, after 29 mo, the fish of the New Brunswick strain were significantly longer (46%; $P < 0.05$) and about twice as heavy as the Newfoundland fish (Fig. 2). Correspondingly, the faster growing New Brunswick strain had lesions

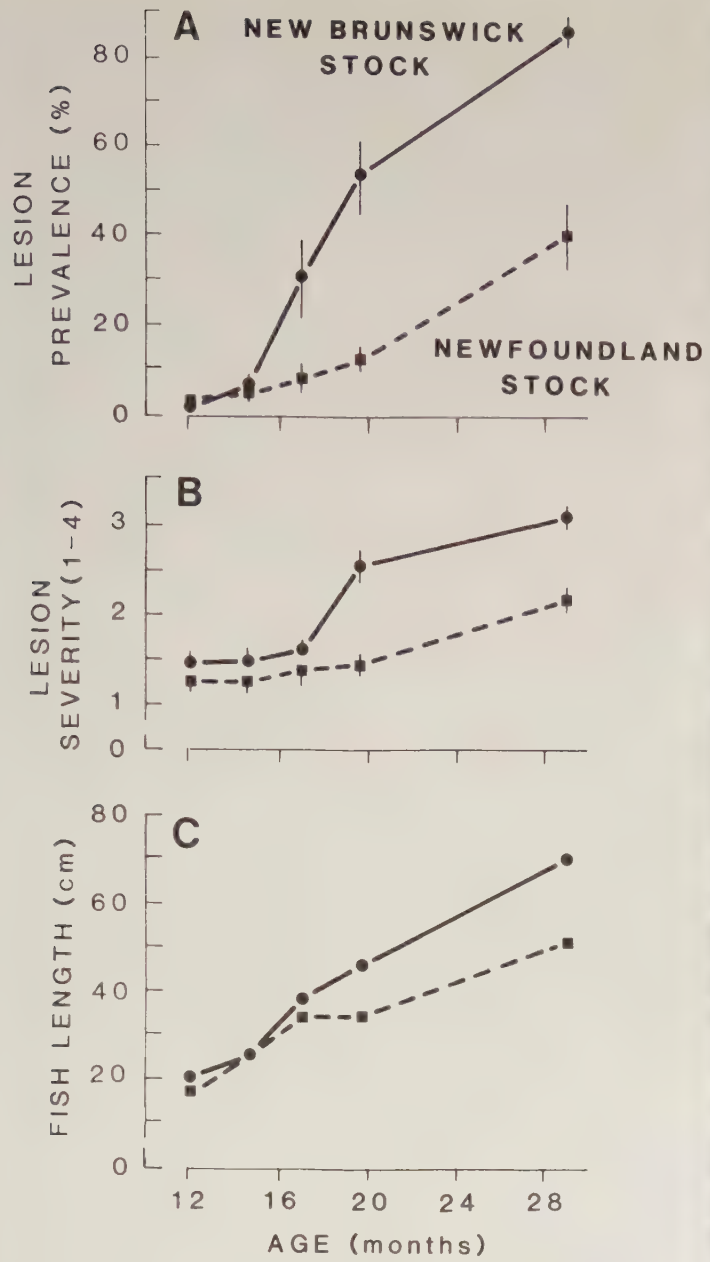

FIG. 2. Comparison of (A) lesion prevalence, (B) lesion severity, and (C) fork length as a function of fish age in a Newfoundland stock (Western Arm Brook) and a faster growing New Brunswick (Saint John River) stock of Atlantic salmon. Each point represents a mean value (bar = SEM) for 7–42 fish.

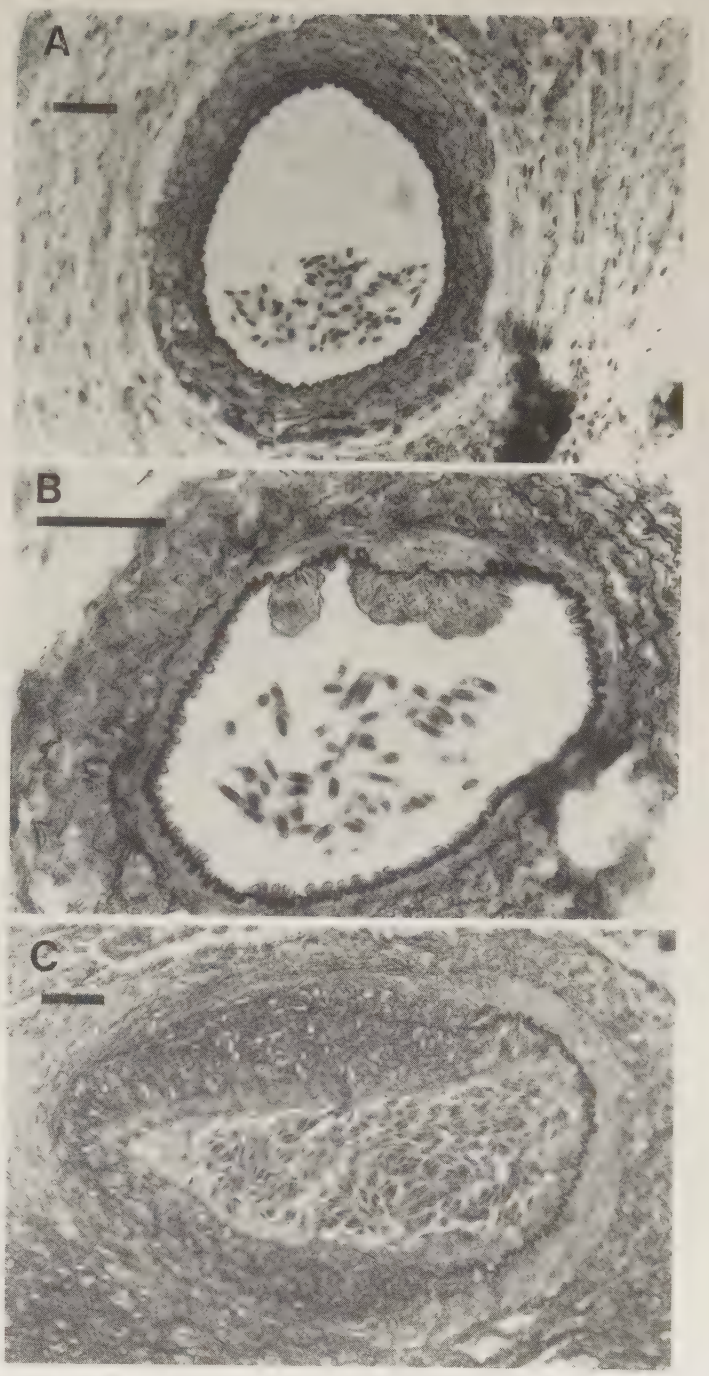

FIG. 3. Cross sections of coronary arteries of Atlantic salmon. (A) Normal artery; (B) small arteriosclerotic lesion; (C) large lesion which partially occludes the vessel lumen. Bars = 50 μm.

which were significantly more prevalent (213%; $P < 0.05$) and more severe (41%; $P < 0.05$) at a given age than the slower-growing Newfoundland fish (Fig. 2).

Discussion

This comprehensive study of the progression of coronary arterial lesions in all life stages of wild and cultured Atlantic salmon confirms that proliferation of smooth muscle cells and development of lesions in the coronary artery of this species begins in juveniles in freshwater, accelerates during the rapid marine growth phase, and reaches severe conditions before attainment of sexual maturity (Farrell et al. 1986; Saunders and Farrell 1988). Since most observations of coronary lesions in salmonids have been in sexually mature individuals, sexual maturation has been suggested as the primary factor associated with arteriosclerosis. However, this and an earlier study (Farrell et al. 1986) clearly demonstrate that coronary lesions have their beginning and may be well developed in immature Atlantic salmon.

Coronary arteriosclerosis was not found in wild or cultured fry (2–6 mo old, 4–7 cm long) but was found in a small percentage of slightly older, larger parr (6–12 mo old, 8–10 cm long) where the prevalence (<1%) and severity (<1.2) were low (Fig. 1). In contrast, lesions developed rapidly during the 1- to 4-yr marine phase when growth increased rapidly. Our present data, based on wild and cultured salmon and cultured strains of salmon with rapid and slow marine growth, show that

progression of lesions accelerates remarkably in parallel with, and possibly in response to, those factors responsible for rapid growth during the marine phase of the life cycle.

What are some conditions responsible for or associated with rapid growth that could also influence progression of coronary lesions? The hypothesis most often suggested is that diet influences development of coronary arteriosclerosis (Moore et al. 1976). Indeed, dietary cholesterol can promote an increase in lesion prevalence in Atlantic salmon (Farrell et al. 1986). Moreover, plasma cholesterol and low-density lipoproteins are naturally high in maturing salmonids (Farrell and Munt 1983). Lesion proliferation in salmonids can also parallel natural (Eaton et al. 1984) or experimental increases (Farrell et al. 1986) in plasma low-density lipoproteins. The most obvious changes encountered when salmon smolts leave their nursery streams to begin marine life are increased salinity and a greatly enhanced food supply in both quantity and quality. Oceanic salinity per se (30 ‰) does not enhance growth rate; Atlantic salmon in the postsmolt stage grow as fast or faster in fresh than in seawater, provided adequate food is available (Saunders and Henderson 1969). The type of fatty acids available in the freshwater stream habitat and in the sea is of special interest, particularly since increased levels of ω-3 fatty acids are considered a beneficial dietary intervention for cardiovascular disease in humans. Juvenile Atlantic salmon in their nursery streams feed largely on aquatic insects in which the predominant polyunsaturated fatty acid is of the ω-6 type, i.e. arachadonic acid (Ackman and Takeuchi 1986). Marine fishes which comprise a large portion of the salmonid diet are rich in the ω-3 types, i.e. eicosapentaenoic and docosahexaenoic acids (Ackman et al. 1988). It is common practice to use marine oils (rich in ω-3 fatty acids) (Ackman et al. 1988) as a dietary lipid source in feeds for cultured salmon, and these fish develop lesions. Cultured juvenile salmon in the present study did not have a greater prevalence or more serious coronary lesions at given sizes than their wild counterparts. Moreover, the present authors, together with Santosh P. Lall (Department of Fisheries and Oceans, Halifax, N.S.) and Robert G. Ackman and Sherilyn Polvi (Technical University of Nova Scotia, Halifax), have conducted a 6-mo feeding trial using diets rich in ω-3 or ω-6 fatty acids. Although postsmolt salmon on the different diets grew satisfactorily, the unpublished results of this study provided no evidence that diets enriched with marine fish oils led to any changes in the progression of coronary lesions. Therefore, although marine fishes have high levels of tissue ω-3 polyunsaturated fatty acids (Ackman and Takeuchi 1986; Gong and Farrell 1990), it appears that the abrupt shift from a high ω-6 to a high ω-3 diet has no major impact on lesion development and that, moreover, the high dietary ω-3 intake does not appear to prevent the rapid development of coronary lesions in Atlantic salmon. Why the beneficial effects of ω-3 fatty acids observed in humans are not observed in fishes is an enigma. A possible explanation is that the biosynthetic pathways in fishes are such that ω-3 fatty acids are not incorporated in the appropriate phospholipids for synthesis of prostaglandins, leucotrines, and thromboxanes that tend to minimize lesion formation.

While we can exclude sexual maturation per se as the primary factor in the etiology of coronary lesion formation in Atlantic salmon (Farrell et al. 1986), we cannot exclude the possible effects of the long-term endocrinological aspects of sexual maturation and other hormonal changes associated with marine growth. These may offer some clues as to growth-related progression of coronary lesions. Hunt et al. (1982) reported that growth in weight of sexually maturing male Atlantic salmon was greater during late spring (March–May) than in nonmaturing fish and suggested that low levels of the sex hormones 11-oxotestosterone and testosterone have an anabolic effect. These findings suggest that endocrinological changes associated with sexual maturation — not maturation itself — may provide potent stimulation of growth at any time during the marine phase and that these changes might be related to the rapid progression of coronary lesions during that period.

Farrell et al. (1986) and the present study showed that coronary arteriosclerosis is already developed in Atlantic salmon smolts. Kubasch and Rourke (1990) suggested, from their study of steelhead trout, that the smolting process might be a key event in the etiology of coronary artery disease in salmonids. Parr–smolt transformation represents a major transition between riverine life in the salmon's nursery stream and marine life. Recent evidence shows that smolting begins during the last summer–autumn of river life (Thorpe 1977; Thorpe et al. 1980; Bailey et al. 1980; Kristinsson et al. 1985). The incipient smolts begin and maintain a period of rapid growth during late summer – autumn. The result is development of upper and lower modal groups based on length–frequency. The upper modal fish become smolts the following spring, while the lower modal fish remain in freshwater another year before smolting. Smolting has been compared with metamorphosis, as in amphibians, marking the transition between life stages which differ anatomically, physiologically, and behaviorally (Dickhoff and Darling 1983; Specker 1988). Parr–smolt transformation is associated with notable changes in levels of several hormones: thyroid (Dickhoff and Sullivan 1987; McCormick et al. 1987), growth hormone (Boeuf et al. 1989; Björnsson et al. 1989); prolactin (Prunet and Boeuf 1989), and corticosteroids (Specker 1982). Thyroid and growth hormones have anabolic effects. Although development of salinity tolerance is a prime requisite of a smolt entering the sea, the metabolic changes associated with smolting (Dickhoff and Sullivan 1987; McCormick and Saunders 1987) appear to set the stage for the period of rapid growth, commencing with establishment of length–frequency bimodality in the autumn and continuing during and after completion of smolting and migration to the sea the following spring.

The profile of metabolic adjustments associated with smolting includes carbohydrate reserves and usage, amount and type of lipid stored and used, lipid-moisture dynamics, oxygen consumption, protein metabolism, and activity of respiratory enzymes (for reviews, see Woo et al. 1978; Ackman and Takeuchi 1986; Sheridan 1989; Farmer et al. 1978; Higgins 1985; McCormick et al. 1989). An hypothesis that progression of coronary arterial lesions is related to the metabolic adjustments that develop in association with smolting and leading to rapid growth in marine environment is worthy of further study.

Another hypothesis to account for progression of coronary lesions in association with rapid growth is a response to vascular injury as seen in mammals (Ross and Glomset 1973; Ross 1984). Vascular injury may arise in salmonids because the coronary artery lies on the surface of the highly compliant bulbus anteriosus and ventral aorta. Overdistention of the ventral aorta and bulbus arteriosus as a result of acute hypertension would physically disturb the coronary artery, altering its blood flow pattern and perhaps causing vascular injury. Acute hyperten-

sion as a result of systemic vasoconstriction (Jones and Randall 1978) occurs when circulating catecholamines are elevated, and a flight or fight mobilization of blood-borne catecholamines is a characteristic response of fishes to natural stresses (Mazeaud and Mazeaud 1981). Thus, it is likely that natural stresses can lead to hypertension which, in turn, disrupts the coronary artery. The correlation between lesion development and growth, or growth rate, may reflect indirectly the sum of stressful events in the salmon's life.

Since coronary arteriosclerotic lesions in the salmonids studied do not contain intra- or extracellular lipid or calcium accumulations that characterize the plaques of mammalian atheroma (Robertson et al. 1961; House and Benditt 1981; Farrell et al. 1986), one might argue that the rather uncomplicated accumulations of smooth muscle cells are one consequence of the ageing process. These lesions, if they are indeed lesions, are similar to those found in early stages of mammalian arteriosclerosis (House and Benditt 1981). That salmonid coronary lesions progress so rapidly is an enigma, since salmonids possess high levels of ω-3 fatty acids (Ackman et al. 1988) which suppress intimal smooth muscle proliferation in mammals by decreasing the production of endothelial-derived growth factors (Fox and DiCorleto 1988). Perhaps the high levels of ω-3 fatty acids and high proportions of high-density lipoproteins in salmonids (Eaton et al. 1984; Farrell et al. 1986) are responsible for the absence of lipid deposits in salmonid coronary lesions. In view of the similarity of vascular smooth muscle proliferation in coronary lesions of salmonids and mammals and the clear relationship between growth rate and progression of salmonid lesions and intensity of growth, these fishes are an attractive model for studies of arteriosclerosis and ageing.

In conclusion, our study of coronary arteriosclerosis in all life stages of wild and cultured Atlantic salmon shows that both prevalence and severity of lesions increase directly with growth rate. Lesions appear first in juvenile salmon in freshwater; smolts entering seawater have a low prevalence of mild lesions. Rapid growth of salmon in the marine environment is accompanied by a dramatic increase in lesion development. Wild and cultured salmon, although at different ages at comparable sizes, have similar prevalence and severity of lesions. In two strains of cultured salmon growing at different rates, the faster growing strain had greater lesion development. These results support our hypothesis that coronary lesions in salmonids are promoted by growth or factors associated with growth. We conclude that lesions develop as a consequence of somatic growth or metabolic processes associated with growth, rather than as a function of age.

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Influence of Water Clarity on the Catchability of Six Freshwater Fish Species in Bottom Trawls

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Bottom trawl surveys are carried out every autumn to estimate the relative abundance of six major fish species, especially that of pikeperch (*Stizostedion lucioperca*) and Eurasian perch (*Perca fluviatilis*), in the 182 000-ha Lake IJssel, The Netherlands. The catchability of these species is influenced by light intensity at the bottom and therefore by water clarity and water depth. In autumn, water clarity can differ greatly from day to day because of wind-mediated resuspension of sediments. Catchability of ruffe (*Gymnocephalus cernua*) and age 0 pikeperch showed a significant inverse relationship with light intensity at the bottom, and therefore, a correction should be made when catch data for these species are used to estimate population size or year-class strength. Results were not consistent for perch, while for smelt (*Osmerus eperlanus*), roach (*Rutilus rutilus*), and bream (*Abramis brama*) the influence of light intensity on catchability was not significant. Corrected and uncorrected estimates of the abundance of age 0 pikeperch, based on trawl samples, were compared to demonstrate the effect of water clarity on the estimation of year-class strength. Increased water clarity can lead to zero catches and consequently diminish the effectiveness of sampling programmes.

Des campagnes d'échantillonnage au chalut de fond visant à estimer les abondances relatives de six espèces importantes de poissons, particulièrement celle du sandre (*Stizostedion lucioperca*) et de la perche (*Perca fluviatilis*), sont effectuées chaque automne dans le lac IJssel, Pays-Bas, d'une superficie de 182 000 ha. La possibilité de capture de ces espèces est fonction notamment de l'intensité lumineuse dans le fond et donc de la limpidité et de la profondeur de l'eau. À l'automne, la limpidité de l'eau peut varier considérablement de jour en jour en raison du brassage des sédiments sous l'action du vent. La possibilité de capture du sandre d'âge 0 et de la grémille (*Gymnocephalus cernua*) variait en raison inverse de l'intensité lumineuse dans le fond; ainsi, un facteur de correction doit être appliqué quand on utilise des données de prises pour évaluer la taille des populations ou des classes d'âge. Aucune tendance n'a pu être dégagée dans le cas de la perche tandis que l'influence de l'intensité lumineuse sur la possibilité de capture de l'éperlan d'Europe (*Osmerus eperlanus*), du gardon (*Rutilus rutilus*) et de la brème (*Abramis brama*) n'était pas significative. On a comparé les estimations corrigées et non corrigées de l'abondance des sandres d'âge 0, fondées sur des échantillons recueillis au chalut, pour démontrer l'effet de la limpidité de l'eau sur l'estimation de la taille de cette classe d'âge. Une limpidité de l'eau élevée peut réduire les captures à néant et donc diminuer l'efficacité des programmes d'échantillonnage.

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Bottom trawling is a well-known technique for sampling fish stocks (e.g. Sissenwine et al. 1983). Catches are usually standardised as catch per unit effort (CPUE). Catchability or gear efficiency, however, can vary with environmental conditions; thus, temperature influences swimming performance (Wardle 1983), and water clarity can influence the vertical distribution of fish (Bohl 1980) or trawl visibility (Glass and Wardle 1989). Much attention is given to fish behaviour in relation to towed fishing gear (Wardle 1983), and marine cinematographic studies have revealed that sight is the most important factor in gear detection by fish (Glass and Wardle 1989).

The effects of varying light conditions on the catchability of fish species have seldom been quantified and experiments are mostly limited to the comparison of day and night hauls (Butterworth and Coles 1977; Quinn and Kojis 1987). Differences in catch rates are often due to changes in the vertical distribution of fish (e.g. Atkinson 1989; Bohl 1980). Catchability can decrease at night ($<10^{-6}$ lx) because the herding effect of the sweeps of the trawl disappears, and during daytime because of early detection of the gear at high light levels (>1 lx) (Glass and Wardle 1989). Thus, midwater trawl efficiency for sockeye salmon (*Oncorhynchus nerka*) during night hauls was inversely related to ambient light, varying with lunar phase and cloudiness (Robinson and Barraclough 1978). Lower water clarity, during day hauls, is thought to have increased CPUE threefold in trawls of yellow perch (*Perca flavescens*) in Oneida Lake (Nielsen 1983). Light conditions during the day

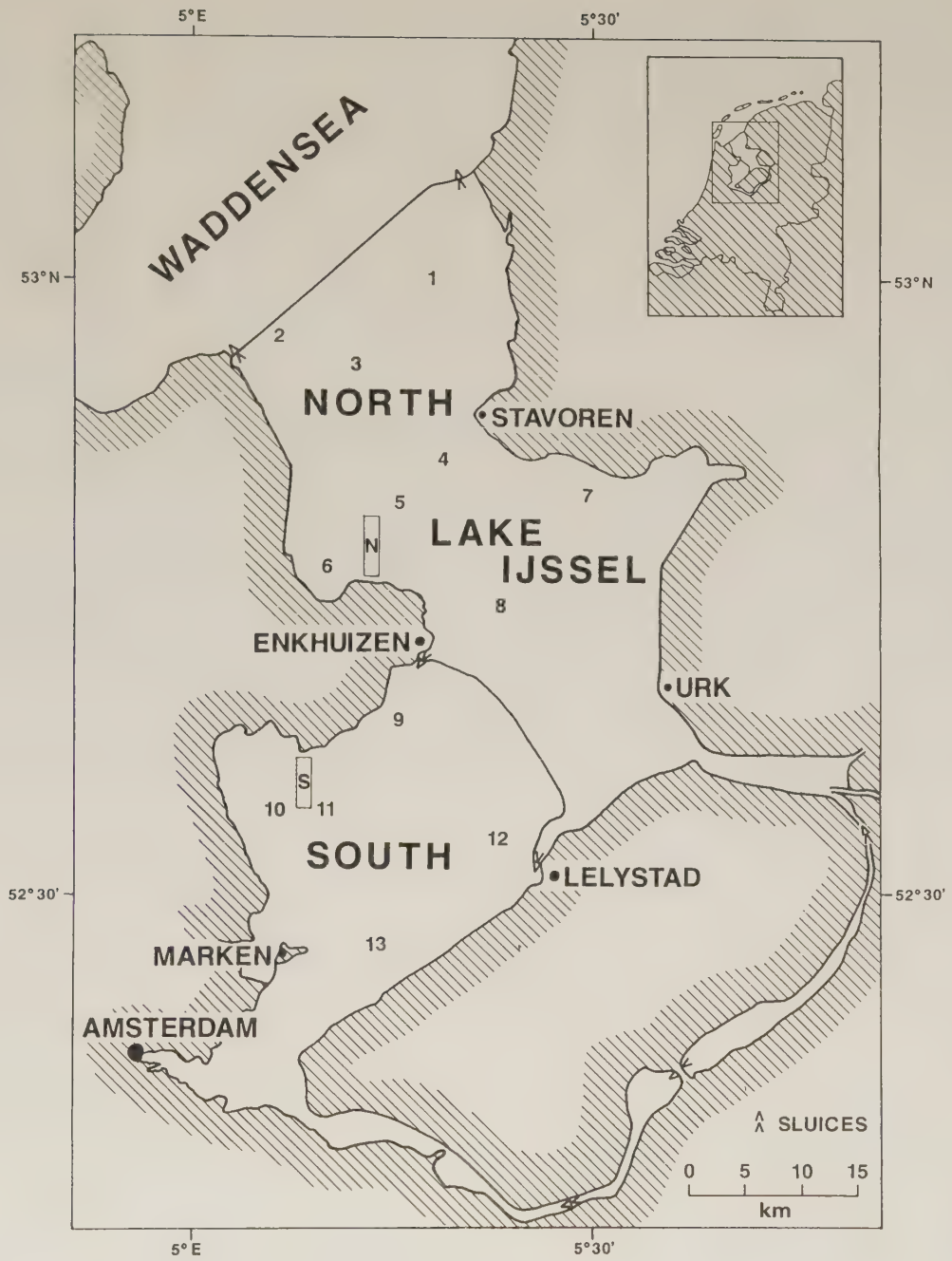

FIG. 1. Netherlands Institute for Fisheries Research Bottom trawl sampling areas Wagenpad (N) and Hoornse Hop (S) at Lake IJssel and regular stations of the lakewide survey (1–13), which was carried out by the Department of Coastal and Inland Fisheries and Cultures, Ministry of Agriculture and Fisheries.

can vary considerably over short periods due to resuspension of sediments by wind (Carper and Bachmann 1984).

Bottom trawls surveys are carried out in Lake IJssel, The Netherlands, every autumn to estimate the relative abundance of six major fish species. Our objective was to study the effects of variations in light conditions near the bottom on catchability of the six major freshwater fish species caught during the day with a bottom trawl. The species concerned were European smelt (*Osmerus eperlanus*), bream (*Abramis brama*), roach (*Rutilus rutilus*), ruffe (*Gymnocephalus cernua*), and especially Eurasian perch (*Perca fluviatilis*) and pikeperch (*Stizostedion lucioperca*).

Materials and Methods

Description of the Area

Lake IJssel is a large and shallow eutrophic lake, formerly an inland sea, divided into a northern (112 000 ha) and southern basin (70 000 ha) by the construction of a dyke in 1975 (Fig. 1). The northern basin has an average depth of 4.5 m,

with depressions up to 10 m deep caused by former tidal movements. The southern basin has an average depth of 3.6 m. Water clarity in both parts varies between 0.1 and 2 m Secchi disk depth, averaging 0.65 m. In both basins, the average water temperature is 10.4°C and varies annually from 0 to 25°C. Total P is 0.22 $mg \cdot L^{-1}$. These and other physicochemical parameters are described in Willemsen (1977).

In Lake IJssel, the influence of wind on water clarity is most pronounced in autumn and winter when algal densities are low. During lakewide survey trawling in the period 1966–87, sampling was started when Secchi disk readings were about 0.8 m or less assuming constant catchability. In years when water stayed clear (over 0.8 m Secchi disk depth), sampling was ultimately carried out irrespective of water clarity, but corrections for the assumed lower catchability could not be made.

Surveys

Two types of bottom trawl surveys are carried out in Lake IJssel in the autumn of every year to estimate the abundance of the major fish species: two selected areas (survey I) and lakewide (survey II).

Survey I (two areas)

A trawl survey was made in two selected areas that were homogeneous in depth and bottom type: "Wagenpad" (N) in the northern part and "Hoornse Hop" (S) in the southern part of Lake IJssel (Fig. 1). Within each area, multiple hauls were made at varying distances parallel to the shore. Data were analysed for 1981–88. Area N was sampled every year, while area S was not sampled in 1984 and 1987.

Under the assumption that within these areas, fishes were homogeneously distributed, quantitative effects of water clarity on the catchability of fish species were estimated using data from this survey.

Area N was sampled within 12 d every year in which 13–27 hauls were made and at distances from the shore varying from 200 to 5400 m. Total depths at the sampling sites ranged from 4.5 to 6.5 m and Secchi disk readings from 0.4 to 1.1 m (Table 1). In area S, similar data were collected in a 2-d period using 8–13 hauls. Distance from shore varied between 500 and 5000 m; total depth ranged between 3.4 and 4.0 m and Secchi depth between 0.25 and 0.70 m (Table 2). In area S, Secchi depth readings did not vary in 1981 and in 1982; therefore, these years were omitted from the analyses. The start of the first and last trawl haul was at 08:35 and 15:45, respectively. The ratio between total water depth and Secchi disk readings (TS ratio) varied between 5 and 17 (Fig. 2).

Survey II (lakewide)

From 1966 until 1989, a lakewide trawl survey covering eight stations in the northern part and five stations in the southern part of Lake IJssel was made (Fig. 1). At each station, a single haul was taken. Data from this survey are used here to dem-

TABLE 1. Station location and catch characteristics (min., mean, max.) of bottom trawls at area N during 1981–88. n = number of hauls; gr = age-group; date = first and last day of sampling; time = earliest and latest hour of sampling; blanks = no data.

Year	n		Date	Time	Distance from shore (m)	Water depth (m)	Secchi depth (m)	Perch gr 0 (n)	Perch gr 1 (n)	Perch gr ≥ 2 (n)	Pike-perch gr 0 (n)	Ruffe (kg)	Smelt (kg)	Roach (kg)	Bream (kg)
1981	13	Min.	12 Oct.	08:34		5.5	0.40	5	56	18	1	1.0	5.5	7.7	1.3
		Mean		11:47		5.8	0.58	21	208	49	19	7.4	27.8	26.0	54.7
		Max.	22 Oct.	15:15		6.0	0.85	43	681	120	49	23.1	66.5	56.1	192.0
1982	20	Min.	11 Oct.	09:00		5.5	0.40	13	0	6	4	0.1	8.6	0.6	0.1
		Mean		12:22		5.7	0.66	38	5	77	25	2.0	20.8	37.7	21.1
		Max.	14 Oct.	15:45		6.0	0.80	97	18	197	104	8.6	33.0	73.2	59.5
1983	17	Min.	18 Oct.	09:04		4.5	0.40	178			2	0.6	4.8	6.9	1.8
		Mean		12:42		5.8	0.70	505			116	7.0	12.8	38.2	31.9
		Max.	27 Oct.	14:39		6.3	0.90	2412			497	59.1	23.8	81.3	66.6
1984	25	Min.	22 Oct.	08:45	200	4.5	0.60	1	4	0	2	0.1	0.3	2.3	0.0
		Mean		12:03	2090	5.9	0.84	58	71	60	21	1.0	10.4	37.2	63.0
		Max.	1 Nov.	15:19	5400	6.5	1.00	134	185	183	64	4.2	40.1	81.0	172.0
1985	20	Min.	28 Oct.	08:40	350	5.3	0.60	152			1	0.2	2.1	3.2	0.9
		Mean		11:45	1999	5.8	0.83	1742			5	6.2	7.4	32.5	68.8
		Max.	8 Nov.	14:55	4000	6.5	1.10	5072			12	29.8	17.7	110.0	225.0
1986	27	Min.	27 Oct.	08:49	250	5.2	0.40	402	213	5	3	4.9[a]	5.1[a]	2.0	6.9
		Mean		11:31	1957	6.0	0.49	900	924	37	16	13.5[a]	14.1[a]	29.2	91.1
		Max.	6 Nov.	15:19	4000	6.5	0.70	1564	1493	71	49	22.0[a]	29.8[a]	100.0	405.0
1987	19	Min.	26 Oct.	08:55	250	5.5	0.50	51	251	37	0	0.5	3.5	0.7	1.5
		Mean		12:00	1684	5.9	0.72	140	881	386	5	13.9	18.0	19.7	59.9
		Max.	29 Oct.	14:34	3500	6.2	0.95	310	2072	533	13	24.9	39.9	46.3	485.0
1988	26	Min.	31 Oct.	09:10	250	5.0	0.60	13	1	43	1	1.0	1.9	0.8	0.0
		Mean		12:10	1837	5.8	0.79	68	9	228	11	11.9	8.6	7.6	19.9
		Max.	3 Nov.	14:55	4000	6.2	1.00	145	24	439	30	35.0	17.0	26.0	163.4

[a]Ruffe and smelt catches in area N in 1986 were separated only for 11 out of the 27 hauls. A subset of these 11 hauls was used to standardise the independent variables for use in the multiple regression analysis of the effects of the independent variables on the catches of those two species.

TABLE 2. Station location and catch characteristics (min., mean, max.) of bottom trawls at area S in 1983, 1985, 1986, and 1988. n = number of hauls, gr = age-group; date = first and last day of sampling; time = earliest and latest hour of sampling; blanks = no data.

Year	n		Date	Time	Distance from shore (m)	Water depth (m)	Secchi depth (m)	Perch gr 0 (n)	Perch gr 1 (n)	Perch gr ⩾ 2 (n)	Pike-perch gr 0 (n)	Ruffe (kg)	Smelt (kg)	Roach (kg)
1983	8	Min.	20 Oct.	09:40		3.8	0.40	23	150	5	2	3.4	1.1	1.4
		Mean		12:30		3.9	0.44	79	356	18	20	9.0	2.4	8.5
		Max.	21 Oct.	15:04		4.0	0.50	145	519	36	62	15.9	3.3	25.1
1985	10	Min.	6 Nov.	09:04	500	3.4	0.30	155	23	22	2	7.0	5.3	1.6
		Mean		10:59	2750	3.8	0.34	279	75	63	10	10.8	9.7	7.0
		Max.	7 Nov.	13:55	5000	4.0	0.35	408	159	97	17	18.3	18.1	13.2
1986	10	Min.	28 Oct.	09:19	500	3.7	0.25	47	156	35	11			2.1
		Mean		11:55	2750	3.9	0.30	426	195	59	16			5.5
		Max.	29 Oct.	14:30	5000	4.0	0.35	967	292	103	31			8.5
1988	13	Min.	9 Nov.	09:45	500	3.6	0.40	4	1	19	0	4.4	1.6	0.8
		Mean		12:30	2385	3.8	0.48	47	5	49	3	17.7	3.9	2.6
		Max.	10 Nov.	15:10	4000	3.9	0.70	134	12	74	7	36.2	7.3	6.5

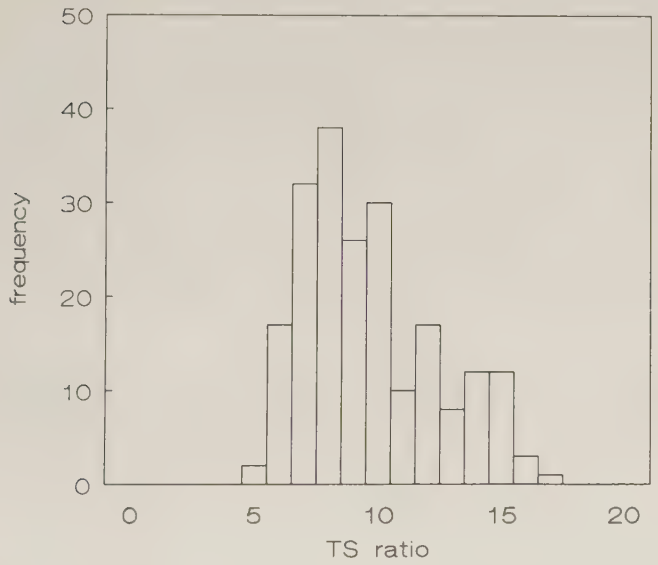

FIG. 2. Frequency distribution of the TS ratio in survey I (n = 208).

onstrate the problem of varying catchability as a consequence of variations in water clarity in general and to show the effect of correcting catches for variations in catchability on the estimation of the year-class strength (YCS) of pikeperch.

During both surveys the abundance of the commercially important perch and pikeperch was indexed by the mean number of age 0 fish caught per standard haul, but the weights of the catches of other species were also recorded. Trawling was carried out with a bottom trawl (cod-end 20-mm stretched mesh, upper rope 13 m, bottom rope 14.75 m). The trawl was kept open with an 8-m broad beam and two 1-m-high danlenos and pulled at an average speed of 1.6 ± 0.1 $m \cdot s^{-1}$. Haul duration was 10 min. For the lakewide survey, haul duration varied in the early years between 15 and 45 min, but was standardised at 10 min from 1982 onwards. Only day hauls were made. Night hauls were impossible because commercial gill nets and fyke nets could not be avoided.

The fish were sorted by species on deck and weighed to the nearest 0.1 kg. To distinguish age 0 pikeperch and age 0 and 1 perch from their length frequency distribution, individual perch and pikeperch were measured to the nearest centimetre total length.

Data Treatment and Analysis

The analysis of data collected with survey I comprised three procedures: transformation of data, multiple regression analysis to determine which indicator variables contributed significantly to catch variation, and construction of a model for correcting catches for the effects of water clarity on catchability, which had lakewide applicability.

Although sampling areas N and S were regarded as homogeneous with respect to fish distribution during the survey, and mortality during the survey was assumed to be negligible, distance from shore and chronological haul number (during one survey within one area) were also included in the analyses. Time of day was included to investigate the influence of incoming light and is expressed as the sine of the angle of the sun altitude. Data are lacking on distance to the shore for area N during 1981–83 and for area S in 1983. These years were therefore not included in the multiple regression analysis. They were included for the construction of a model which corrects catches for effects of water clarity.

In this study the effect of within-year variations in water clarity and other independent variables on the bottom trawl catches is analysed. To generate a model which uses data from various years, both the dependent and the independent variables were standardised for their "within-year mean" because year-to-year variations in fish abundance caused by, for example YCS variations would otherwise obscure the effect of the variables on the catches. Results will thus express the relative effect of water clarity on the catchability and can be used to calculate indices of fish abundance at a reference water clarity.

Catches from areas N and S were transformed to the natural log of the ratio of the catch from one haul to the geometric mean catch of all hauls within the area in the same year:

$$(1) \quad Cs_{ij} = \log_e (C_{ij}/C_{\cdot j})$$

where Cs = standardised catch, for mean abundance per year, which is log-transformed, C = catch in number (perch and pikeperch) or kilograms (other species) per 10-min trawl haul, and i = haul number in year j.

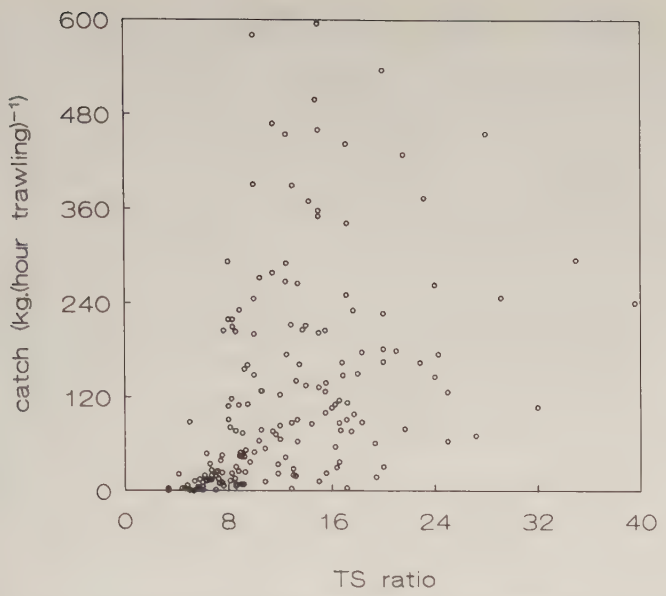

FIG. 3. Trawl catches of ruffe versus the TS ratio during survey II at the northern part of Lake IJssel, 1966–89 (n = 170).

Following Pennington (1983), data were first divided into zero and nonzero catches. Only nonzero values (1066 of 1075 sets for all species) were used for parameter estimation. Bream catch data for area S were not analysed, since they comprised 25% zero catches.

Light intensity at the bottom, where the trawl sampled, was not measured directly and is expressed relative to the incident daylight intensity at the water surface with the ratio between total water depth and Secchi depth, which ratio is further referred to as light intensity. Variations in surface light due to cloudiness at the time of sampling are unknown but assumed to be negligible compared with the effect of varying water clarity. Secchi disk depth can be approximated by the depth of 20% surface light (Lorenzen 1980). Thus the ratio between the depth of a water and Secchi disk depth is the nth power of 0.2^n and indicates the fraction of surface light which reaches the bottom. The total water depth:Secchi depth (TS) ratios were standardised by subtracting the "within-year mean ratio":

$$(2)\quad TSs_{ij} = (T/S)_{ij} - (T/S)_{\cdot j}$$

where TSs = standardised total water depth:Secchi depth ratio, T = total water depth (metres), and S = Secchi depth (metres).

The independent variables "distance to the shore" and "time of day" were similarly standardised by subtracting the "within-year mean." With this standardisation of the variables, data from various years are brought to the same scale (mean = 0) and can consequently be pooled for regression analysis.

To generate a model which corrects catches for varying catchability and which is applicable lakewide, water clarity was the only useful parameter of this study which could be included. Therefore the following model (intercepts were never significant) was calculated:

$$(3)\quad Cs = a \cdot TSs$$

where a = constant.

The estimated parameter a can be used to standardise all data from every year and sampling site, to one TS ratio, through which a better comparison can be made of variations of catch over time and distribution:

$$(4)\quad Cr_{ij} = C_{ij} \cdot e^{a \cdot (TSr - (T/S)_{ij})}$$

where Cr = catch standardised to a reference total water depth:Secchi depth ratio and TSr = reference total water depth:Secchi depth ratio.

The reference total water depth:Secchi depth ratio was calculated as the overall mean of this ratio measured during the lakewide survey.

Results

Catches tend to decline with increasing light intensity at the bottom, as exemplified for catches of ruffe during the lakewide survey (Fig. 3). It is not possible to quantify the effect of light intensity on the catchability of ruffe from these data because catches are a function of local abundance at the sampling sites as well as catchability.

Catches of six species varied largely within the selected areas N and S (Tables 1 and 2). Within years, the minimum variation was less than fivefold for all species except bream, and the maximum variation was more than 100-fold for all species except for age 1 perch. Among years, catches varied more than 100-fold for age 2 and older perch, age 0 pikeperch, ruffe, smelt, and roach and more than 1000-fold for age 0 and 1 perch and bream.

To compare the significance of the independent variables on standardised catches, one correlation matrix was calculated for each fish category. Catches increased with decreasing light intensity at the bottom, as shown by the significant positive correlation between catches and the TS ratio, for all fish categories except for roach, bream, and age 2 and older perch in area N (Table 3) and for roach in area S (Table 4). The significant correlation between catches per fish category and distance to shore showed that in area N, age 1 and older perch and ruffe and roach were common farther offshore, while age 0 pikeperch, smelt, and bream were common farther inshore. Also in area S, smelt catches were highest inshore. No negative trend was found in the catches during the sampling period, except for smelt and age 2 and older perch, so mortality during the survey periods was not manifest. Catches did not correlate with time of day. Therefore, within the period of a day that trawls were made, effects of varying light conditions due to the changing altitude of the sun on the catchability were negligible.

Correlations among the independent variables showed a significant negative correlation between the TS ratio and haul number in area N, which is due to the sampling strategy of starting when circumstances were favourable. A spurious correlation was found between time of day and distance from shore in area N.

Using only those independent variables which were significant ($p < 0.05$) in a multiple regression, 6% (roach) to 31% (age 0 pikeperch) of the variance in catch data could be explained for area N and 18% (age 2 and older perch) to 53% (age 1 perch) for area S (Table 5). Only light intensity and distance from shore contributed considerably to the explanation of the variation in the catches in areas N and S.

Correction factors for catches made during the lakewide survey could only be calculated using the TS ratio, which varied between similar ranges both inshore and offshore. The effect of distance from shore on the catches cannot be extrapolated lakewide (Fig. 1). It is realised that by keeping light intensity

TABLE 3. Correlation matrix of the standardised catches of six fish species with the TS ratio (TS), distance from shore (DI), chronological haul number (HANO), and time of day (TI) at area N (n = number of hauls; $*p < 0.05$; $**p < 0.01$).

Species	Age-group	n	TS	DI	HANO	TI
Perch	0	117	0.287**	−0.027	0.035	−0.090
Perch	1	97	0.220**	0.215*	−0.102	−0.022
Perch	≥2	96	−0.058	0.474**	−0.032	−0.120
Pikeperch	0	115	0.522**	−0.221*	−0.157	0.155
Ruffe	All	100	0.399**	0.213*	0.017	−0.163
Smelt	All	101	0.281**	−0.257**	−0.084	0.173
Roach	All	117	0.146	0.247*	−0.023	−0.157
Bream	All	115	0.179	−0.323**	−0.051	−0.013
DI		117	−0.099			
HANO		117	−0.400**	0.043		
TI		117	−0.013	−0.424**	0.110	

TABLE 4. Correlation matrix of the standardised catches of five fish species with the TS ratio (TS), distance from shore (DI), chronological haul number (HANO), and time of day (TI) at area S (n = number of hauls; $*p < 0.05$; $**p < 0.01$).

Species	Age-group	n	TS	DI	HANO	TI
Perch	0	33	0.707**	0.220	−0.186	0.297
Perch	1	33	0.642**	0.316	−0.456**	0.080
Perch	≥2	33	0.421**	0.264	−0.280	−0.025
Pikeperch	0	30	0.524**	0.180	−0.251	−0.186
Ruffe	All	23	0.577**	0.066	−0.272	0.295
Smelt	All	23	0.479*	−0.423*	−0.439*	−0.088
Roach	All	33	0.120	0.178	−0.270	−0.295
DI		33	0.322			
HANO		33	−0.179	0.075		
TI		33	0.264	0.231	0.059	

TABLE 5. Significant ($p < 0.05$) variables explaining variance in catch data of six fish species and the fraction of variance explained within two areas N and S of Lake IJssel. R^2 = fraction variance explained; DI = distance from shore; HANO = chronological haul number; TS = TS ratio.

		Area N		Area S	
Species	Age-group	Variables	R^2	Variables	R^2
Perch	0	TS	0.08	TS	0.50
Perch	1	TS, DI	0.11	TS, HANO	0.53
Perch	≥2	DI	0.22	TS	0.18
Pikeperch	0	TS, DI	0.31	TS	0.27
Ruffe	All	TS, DI	0.23	TS	0.33
Smelt	All	TS, DI	0.13	TS, DI	0.50
Roach	All	DI	0.06		
Bream	All	DI	0.10		

as the only explanatory variable, its effect can be overestimated, if the explanatory capability is incorrectly taken over from the excluded variables.

If the TS ratio is a good indication of catchability, then effects should be similar in both areas N and S. Significant effects of light intensity on catchability were found in both areas for ruffe, smelt, age 0 and 1 perch, and age 0 pikeperch (Table 5). Thus for these species, regression coefficients of fish catches on the TS ratio from both areas could be compared, using eq. (3). To improve the estimates of the effect of light intensity on the catchability, data collected at area N during 1981–83 and at area S in 1983 were also used in this comparison (Table 6). For age 0 pikeperch and ruffe, slopes did not differ significantly between the two areas. Therefore, data could be combined to calculate an overall model for these two species. The negative effect of a higher light intensity near the bottom on the catchability was stronger for age 0 pikeperch than for ruffe (Table 6; Fig. 4). Catches of age 0 pikeperch decreased 22% and those of ruffe 15% as light intensity increased fivefold, i.e. as the TS ratio is decreased by one unit. Slopes of the regression of the catches of both perch age-groups on light intensity differed significantly between the two areas studied (age 0: $p < 0.001$; age 1: $p < 0.01$). With the extended dataset, light intensity no longer had a significant effect on the catches of smelt.

The autumn trawl surveys aim to estimate YCS, in terms of age 0 abundance, of the commercially important pikeperch and

TABLE 6. Regression coefficients (a) and their standard error (SE) of linear models, $\log_e(C) = a \cdot TS$, to correct catch data (C) of four fish species for light intensity at the bottom, expressed as the TS ratio, for the two distinct areas N and S and lakewide (L) in Lake IJssel. (n = number of hauls; R^2 = fraction of variance explained; $^{**}p < 0.01$).

Species	Age-group	Area	n	a	SE	R^2
Perch	0	N	167	0.092	0.028	0.06**
Perch	0	S	41	0.459	0.075	0.49**
Perch	1	N	128	0.052	0.036	0.02
Perch	1	S	41	0.246	0.051	0.38**
Pikeperch	0	N	165	0.244	0.030	0.29**
Pikeperch	0	S	38	0.243	0.089	0.17**
Pikeperch	0	L	203	0.244	0.028	0.27**
Ruffe	All	N	150	0.153	0.042	0.08**
Ruffe	All	S	31	0.269	0.066	0.36**
Ruffe	All	L	181	0.161	0.038	0.09**
Smelt	All	N	151	0.021	0.027	0.00
Smelt	All	S	31	0.116	0.064	0.10

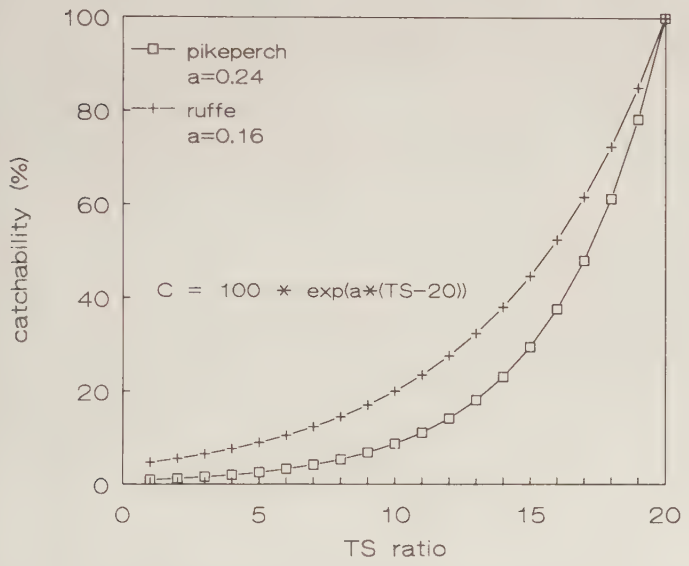

FIG. 4. Modelled relationship between light intensity, expressed as the TS ratio, and catchability (C) of ruffe and age 0 pikeperch in Lake IJssel. A TS ratio of 20 was used as a reference point.

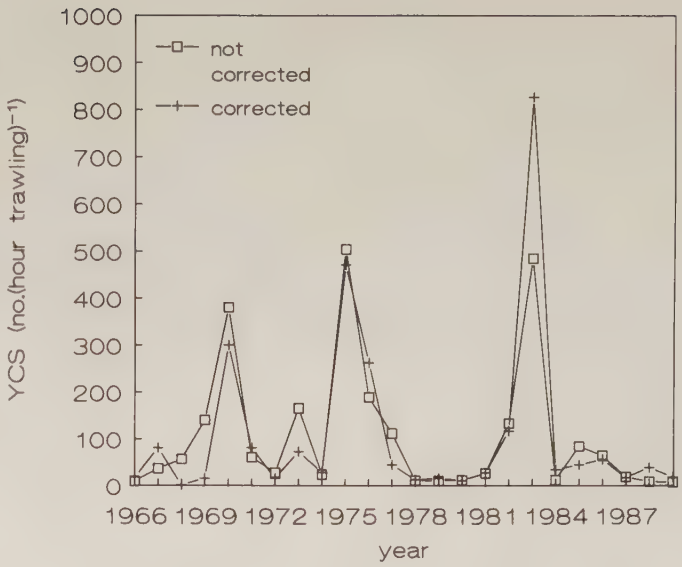

FIG. 5. Uncorrected and corrected pikeperch YCS estimates, indexed as mean number of age 0 fish per hour of trawling during survey II in Lake IJssel, 1966–89. Corrected = standardisation to the mean TS ratio (13.1) over this period for the effect of light intensity on catchability.

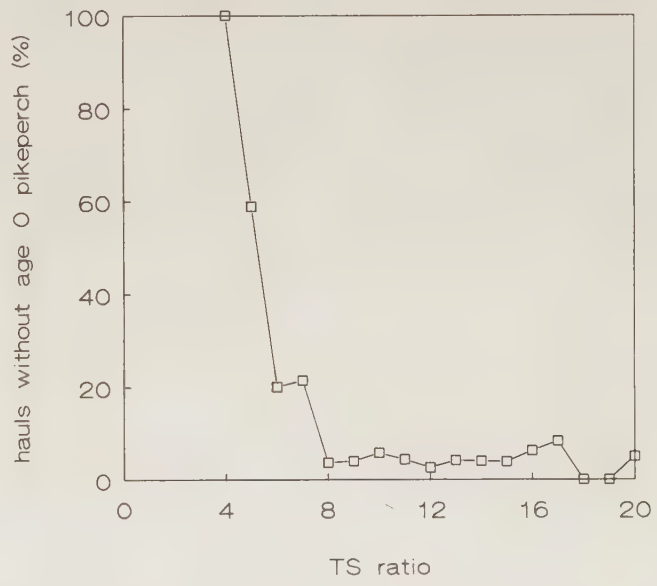

FIG. 6. Percentage of trawl hauls without age 0 pikeperch versus the TS ratio. Data of survey I and II combined. A TS ratio of 20 represents all hauls with a TS ratio ≥ 20. n = 477.

perch. Log-transformed estimates of YCS of perch and pikeperch from the lakewide trawl survey were highly correlated with the log-transformed landings of the same year-classes by the commercial gillnet fishery (perch: $r = 0.91, p < 0.01$ for year-classes 1969–84; pikeperch: $r = 0.69$, $p < 0.01$ for year-classes 1971–86) (unpublished data). Age 0 densities of perch and pikeperch are therefore regarded as good predictors of the yield. Since the effects of light intensity on catches of age 0 pikeperch are the strongest, uncorrected and corrected data of pikeperch were compared for their capability to predict landings. Data were corrected according to eq. (4) using the mean TS ratio over the period 1966–89 during the lakewide survey as reference ratio (TSr = 13.1). Corrected and uncorrected estimates of pikeperch YCS displayed the same fluctuations (Fig. 5). Corrected estimates varied from 98% lower (for 1968) to 349% higher (for 1988) than the uncorrected estimates. The correlations of corrected ($r = 0.61, p < 0.05$) and uncorrected ($r = 0.69, p < 0.01$) estimates with the landings of the commercial gillnet fishery for year-classes 1971–86 were both significant, and regression coefficients were not significantly different from each other.

Catches without age 0 pikeperch became less frequent as the TS ratio increased (Fig. 6). In both surveys, 45 out of 477 hauls caught no age 0 pikeperch. Of these 477 hauls, 93 had a TS ratio ≤7 and 29 hauls out of the 45 with zero catches had a TS ratio ≤7. Thus, 4% of the catches with a TS ratio above 7 and 31% with a TS ratio equal to or below 7 caught no age 0 pikeperch.

Discussion

Light intensity is an indicator of variation of catchability, and distance from shore is an indicator of spatial variation in

abundance of fish within the area investigated. For the lakewide survey, only light intensity could be used to correct for variation in the catches. The effects of light intensity on catchability of pikeperch and ruffe could therefore be possibly overestimated. Thus, equality of regression coefficients of light intensity for one species in one area was tested between estimates from the model based on light intensity data only and from the regression model, which also included distance from shore. For both species and both areas the regression coefficients from both models did not differ significantly. Therefore the model, which uses only light intensity as an independent variable, did not overestimate the effect of light intensity on the catchability of pikeperch and ruffe.

In this study, catchability of fish is expressed as a function of light conditions near the bottom. The TS ratio was used as an index for these light conditions. In our study the TS ratio varied mainly between 6 and 15. The daily total incoming visible radiation on a standard clear day is 3.93×10^6 J·m^{-2} (Goudriaan and van Laar 1978) at 52°45 on November 1, which corresponds with 2.83×10^4 lx. Average transmission in The Netherlands is about 50%. So light conditions at the bottom of Lake IJssel varied between 9×10^{-1} and 4.6×10^{-7} lx. Photographic studies on fish in the waters around Orkney, U.K., showed that the ordered pattern of reaction behaviour to an approaching net, seen at high light levels ($>10^{-3}$ lx), ceased at low light levels ($<10^{-6}$ lx) (Glass and Wardle 1989). Although visual performance of the fish species in our study is not known, probably they are better able to detect the trawl at a TS ratio of 6 than at 15.

The effect of the TS ratio on the catchability of pikeperch and ruffe was similar in areas N and S, which have different depths. The TS ratio may therefore be a general indication of the catchability of these bottom-dwelling species (Eloranta and Eloranta 1978; Nilsson 1979). The effects of the light conditions at the bottom on the catchability of all species were consistently stronger (but never significant, except for perch) in the more shallow area S than in area N. This difference is thought to be due not to the difference in light intensity, but to the noise of the survey vessel. This effect was more pronounced in shallow water and under calm weather conditions.

Temperature influences catchability via the swimming performance of fishes (Wardle 1983). Possible differences in catchability between years caused by temperature were not analysed because data were corrected for within-year mean abundance. The maximum variation in water temperatures recorded during a survey was 2.6°C in 1981. Thus, within-year differences in swimming performance probably do not differ much.

Increased light intensity had a negative effect on the catchability of all species. This effect was strongest for pikeperch and then for ruffe. Effects for perch, which were of similar magnitude to those for ruffe, were not consistent for both areas. Perhaps a more patchy distribution of perch than those of the other species results in a higher variation in the catches. Schooling behaviour and water clarity are highly correlated for yellow perch in Lake Mendota; at higher water clarity the space used by a school is larger because of improved visibility (Hergenrader and Hasler 1968). Data of Nielsen (1983) were recalculated to compare the effect of light intensity on the catchability of yellow perch in Oneida Lake with the effects found for perch in Lake IJssel. Light intensity had a stronger effect (regression coefficient = 0.48) in Oneida Lake than in Lake IJssel, perhaps because the absolute light intensity near the bottom was much higher in Oneida Lake.

The effect of light intensity on the catchability of smelt could not be quantified. Correlations between the catches of smelt and light intensity were significant for 1984–88, but including data from 1981–83 resulted in an insignificant correlation. Probably the vertical migration of smelt in relation to variable light conditions, following their zooplankton food (Dembinski 1971; Nilsson 1979; Northcote and Rundberg 1970), induces an extra source of variation in smelt catches with bottom trawls. Hydroacoustic surveys should be made simultaneously with a trawl sampling programme to register the vertical distribution of the smelt (Burczynski et al. 1987).

The effect of differences in catchability on the estimates of relative YCS of pikeperch over the period 1966–89 is small. This is partly explained by the initial sampling strategy which was to wait for favourable conditions, resulting in small variations in water clarity during surveys. Further, YCS variation for pikeperch in Lake IJssel is so large that it overrides the effects of changes in catchability. However, the changes in catchability from one year to another can lead to serious over- or underestimation of the survival rate of a year-class.

Age 0 pikeperch catches were the most susceptible to changes in water clarity. Zero catches of age 0 pikeperch were mainly due to low catchability, not to low abundance. Because zero catches cannot be standardised for light intensity, they have to be minimised. Therefore, sampling can be optimised by interrupting the programme when water clarity exceeds a certain level and by standardising catches to a reference TS ratio for all nonzero hauls. Our results give an objective means for deciding what fraction of zero values is acceptable.

In Lake IJssel, the TS ratio can vary between 2 and 40 (Willemsen 1977). Large variations in water clarity are also found in other situations. In the Oster Lakes (Federal Republic of Germany), Secchi depths vary between 0.5 and 8 m, which results in a TS ratio of 3.9 and 62, respectively, when related to maximum water depth (Bohl 1980). Mean Secchi depth readings in Oneida Lake varied between 1.6 and 3.9 m, and this corresponds to TS ratios of 1.7 and 4.2 when related to mean water depth (Nielsen 1983).

Differences in light conditions and thus differences in catchability will always be found when samples are taken simultaneously at different depths in the water column. Therefore, conclusions that fish are vertically distributed as determined by trawling at different depths or vertical gillnetting can only be justified provided that experimental studies (Scherer 1976) and echo sounding studies (e.g. Dembinski 1971; Kelso et al. 1974; Northcote and Rundberg 1970; Robinson and Barraclough 1978) prove that vertical positioning of fish is related to light intensity.

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Piscivory, Growth, and Size-Selective Mortality of Age 0 Pikeperch (*Stizostedion lucioperca*)

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Buijse, A. D., and R. P. Houthuijzen. 1992. Piscivory, growth, and size-selective mortality of age 0 pikeperch (*Stizostedion lucioperca*). Can. J. Fish. Aquat. Sci. 49: 894–902.

Year-class strength of pikeperch (*Stizostedion lucioperca*), indexed as the age 0 abundance in trawl surveys, varied 300-fold in the northern part of Lake IJssel, The Netherlands, over the period 1966–89. Both mean length and year-class strength of age 0 pikeperch in November were highly correlated with mean summer temperature. Depending on the environmental conditions, especially water temperature and availability of food, the initially unimodal length frequency distribution of age 0 pikeperch developed into a positively skewed, bimodal or negatively skewed distribution towards the end of the summer. Strong year-classes were characterised by larger mean lengths and a negatively skewed frequency distribution, while weak year-classes were smaller and positively skewed. Stomach contents consisted of zooplankton and macrofauna for the smaller specimens and of age 0 smelt (*Osmerus eperlanus*) for the larger pikeperch (>10 cm). Body energy content increased with fish length, and differences in proximate analysis were more pronounced later in the season. The condition of nonpiscivorous age 0 pikeperch was low and decreased over time, while that of piscivorous pikeperch increased. The onset of piscivory, favoured by high temperatures during summer, has a direct positive effect on the growth and survival of age 0 pikeperch.

De 1966 à 1989, l'abondance des classes d'âge de sandre (*Stizostedion lucioperca*) indexée sur l'abondance du groupe d'âge 0 dans des relevés par chalutage effectués dans le secteur nord du lac IJssel, Pays-Bas, a varié par un facteur de 300. La longueur moyenne du sandre et l'abondance du groupe d'âge 0 en novembre étaient étroitement corrélées à la température moyenne de l'eau en été. Selon les conditions environnementales, surtout la température de l'eau et la disponibilité de proies, la distribution unimodale des fréquences de longueurs des sandres d'âge 0 a évolué vers une distribution étalée vers droit, une distribution bimodale ou une distribution étalée vers la gauche vers la fin de l'été. Une plus grande longueur moyenne et une distribution étalée vers la gauche étaient caractéristiques des importantes classes d'âge tandis qu'une plus petite longueur et une distribution étalée vers la droite l'étaient dans le cas des faibles classes d'âge. Les contenus stomacaux des petits sandres se composaient de zooplancton et de macrofaune, et d'éperlans (*Osmerus eperlanus*) d'âge 0 dans le cas des gros sandres de plus de 10 cm de longueur. L'énergie métabolique augmentait en fonction de la longueur du corps du sandre, tandis que les différences révélées par analyse immédiate étaient plus prononcées plus tard au cours de la saison. L'indice de condition de sandres non ichtyophages d'âge 0 était faible au début, puis a diminué en fonction du temps, tandis que celui des sandres ichtyophages a augmenté. Le début de l'ichtyophagie, favorisé par des températures élevées en été, a une incidence positive directe sur la croissance et la survie des sandres d'âge 0.

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Year-class strength (YCS) of pikeperch (*Stizostedion lucioperca*), indexed as the average number of age 0 fish per standard trawl haul during autumn surveys, varied 20-fold in Lake IJssel, The Netherlands, over the period 1966–75. The abundance of age 0 pikeperch during these autumn trawl surveys was highly correlated with the commercial landings of the same year-classes and therefore can be considered a reliable measure of pikeperch recruited to the fishery (Willemsen 1977). Although the positive influence of high summer temperatures is often considered the principal factor contributing towards strong year-class formation of percids (e.g. Koonce et al. 1977; Willemsen 1983; LeCren 1987), the effect of temperature on survival is rarely discussed (Busch et al. 1975; Clady 1976). According to Hokanson (1977), the physiological optimum for pikeperch is 27.3°C and the growth optimum is 28–30°C. Warm summers in Lake IJssel always resulted in a faster growth of age 0 pikeperch, although optimum temperatures are never reached (Willemsen 1983), which suggests that temperature affects survival indirectly through enhanced growth.

In Lake IJssel, strong year-classes of pikeperch are in general characterised by a mean length of over 14 cm by the end of their first growing season (Willemsen 1977, 1983). Major differences between years in mean length at the end of the first growing season (Willemsen 1983) might be explained by higher summer temperatures, but van Densen (1985) suggested that it was the switch towards a piscivorous feeding mode that enhanced growth. In particular, it was hypothesised that the largest numbers of a cohort would profit from this switch. Size-related differential prey availability may result in the divergences in length over time among individuals in the same age-class (Keast and Eadie 1985) and bimodality (DeAngelis and Coutant 1982). Bimodality in size distributions of one

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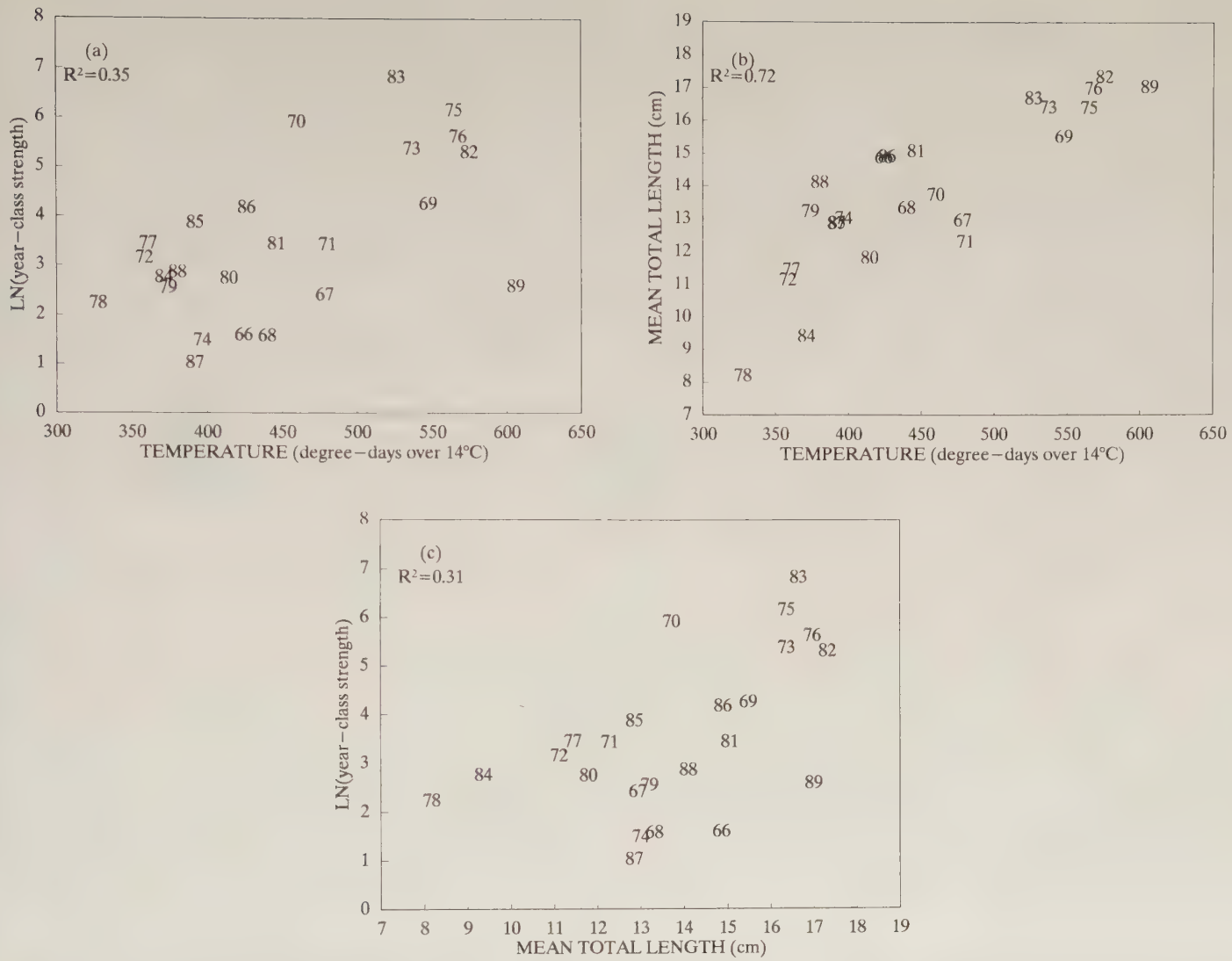

FIG. 1. (a) YCS and (b) mean length of age 0 pikeperch versus water temperature (year-sum of degree-days over 14°C) and (c) YCS versus mean total length based on November trawl surveys in the northern part of Lake IJssel over the period 1966–89. YCS is given as $\log_e$ number caught per hour of trawling (geometric mean of three to eight hauls); $n = 24$; all correlations are significant at $p < 0.01$.

cohort has been described for a number of piscivorous species including largemouth bass (*Micropterus salmoides*) (Aggus and Elliott 1975; Shelton et al. 1979; Timmons et al. 1980), Eurasian perch (*Perca fluviatilis*) (Chodorowski 1975), walleye (*Stizostedion vitreum*) (McIntyre et al. 1987), and pikeperch (van Densen 1985).

The most important prey fish for age 0 pikeperch in Lake IJssel is European smelt (*Osmerus eperlanus*) (Willemsen 1977). Smelt spawn in March and April about 1 mo prior to pikeperch (Willemsen 1977) and therefore have the potential to be readily available as preferred prey for pikeperch when they are large enough to switch to a piscivorous diet. Mean summer temperature had no significant effect on smelt growth in Tjeukemeer (W. Mooij, Waterloopkundig Laboratorium, Rotterdamserveg 185, 2629 HD Delft, The Netherlands, pers. comm.) and Lake IJssel (Brasseur 1990). However, above 15°C the growth rate of smelt was lower than that of planktivorous pikeperch in Tjeukemeer (W. Mooij, pers. comm.). Thus, it seems likely that at high temperatures, planktivorous age 0 pikeperch may surpass age 0 smelt in length and become piscivorous.

Abundance of pikeperch larvae in May and June did not correlate with abundance during autumn trawl surveys (Willemsen 1977). This lack of correlation could be the result of inadequate sampling of larvae or simply because larvae abundance does not determine YCS. The object of this study therefore was to investigate if the switch in feeding behaviour from zooplankton and macrofauna to fish in the summer influences year-class formation of pikeperch. It was hypothesized that when prey fish are not available, smaller pikeperch of the same cohort are in a poor condition resulting in size-selective mortality of the smaller fish. Age 0 pikeperch were therefore analysed for their condition in terms of energy content per unit body weight.

Materials and Methods

Study Area

A description of the chemical and physical characteristics of Lake IJssel, a shallow eutrophic 182 000-ha freshwater lake (52°45′N, 5°20′E), is given in Willemsen (1977) and Buijse et al. (1992). Water temperatures (±0.1°C) in Lake IJssel were measured daily and expressed as degree-days above 14°C.

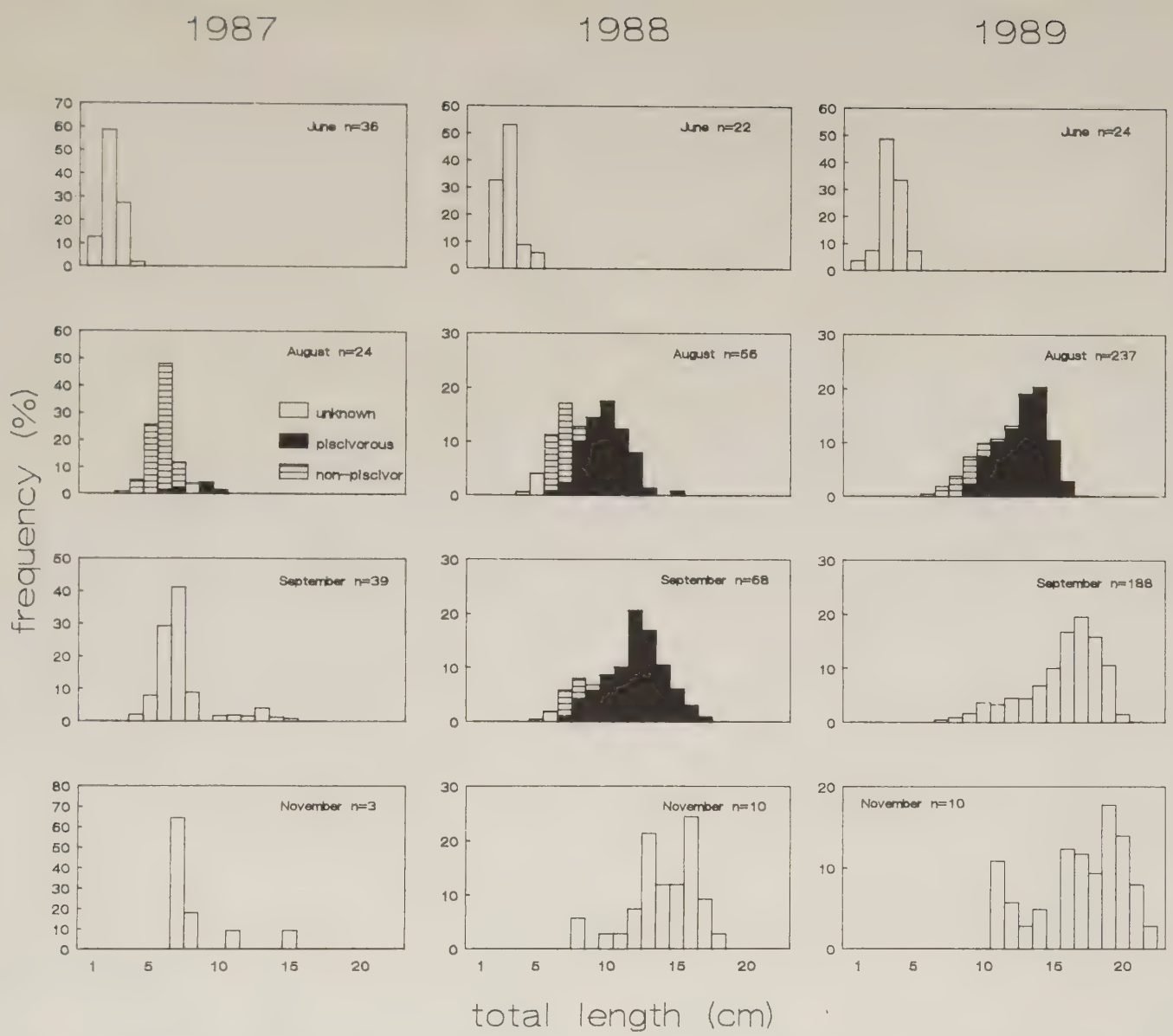

FIG. 2. LF distributions of age 0 pikeperch in the northern part of Lake IJssel in June, August, September, and November 1987–89. Food items per size-class of pikeperch were only recorded in August 1987–89 and September 1988. n = number caught per hour of trawling (geometric mean of 15 hauls of 10 min each).

Surveys

Data used in this study originate from trawl surveys in the northern part of Lake IJssel during 1966–89. A routine lake-wide survey programme was carried out in August and November 1966–86 using a bottom trawl (cod-end 20-mm stretched mesh). A description of the sampling sites and the trawl is given in Buijse et al. (1992). During 1987–89, sampling ws intensified; abundance and length of pikeperch and of smelt were also recorded in June (cod-end 2-mm stretched mesh) and September, and the number of sampling sites was increased to 15 (unpublished data).

Fish were sorted by species on deck and weighed to the nearest 0.1 kg. Total length of individual fish was measured to the nearest centimetre. During surveys in June, age 0 fish were preserved with 4% formalin for later identification and measurement to the nearest 0.5 cm in the laboratory. To estimate the mean abundance of a fish species, indices were calculated by taking the geometric mean of the catches, following the addition of 1 to the catch data. Since the ropes of the 2-mm trawl are about half the length of those of the 20-mm trawl, catches in the 2-mm trawl were doubled to be comparable with catches made by the 20-mm trawl. During the August and September surveys in 1988, age 0 pikeperch were collected and frozen for later determination of their energy content, dry weight, and stomach contents.

Laboratory Methods

Each 1-cm size-class of age 0 pikeperch retained for stomach analysis was weighed to the nearest 0.1 mg and frequencies of occurrence of prey in the stomachs were recorded. Subsequently the fish were cut into 1-cm pieces, freeze-dried, and reweighed. Calorimetric analysis required samples of at least 30 g (wet weight); therefore, the two largest size-classes, 14 and 15 cm in August and 16 and 17 cm in September, were

TABLE 1. Descriptive statistics for age 0 pikeperch, smelt, and water temperature (cumulative degree-days over 14°C) in the northern part of Lake IJssel during four sampling periods in 1987, 1988, and 1989. n = number caught per hour of trawling (geometric mean of 15 hauls of 10 min each); TL = mean total length; SD = standard deviation of the length; SKNS = skewness; $*p < 0.05$, $***p < 0.001$ = significance of skewness.[a]

	Pikeperch				Smelt				
	n	TL (cm)	SD (cm)	SKNS (cm)	n	TL (cm)	SD (cm)	SKNS (cm)	Temperature
1987									
29 June – 3 July	36	2.2	0.66	0.17	3 632	3.6	0.57	−0.17	46
17 – 21 August	24	6.0	1.17	0.98***	3 561	6.2	0.66	−0.19	256
7 – 10 September	39	7.2	2.06	1.96***	1 607	6.8	0.50	−0.43	342
9 – 12, 17 November	3	8.3	2.48	2.21***	378	7.7	0.72	−0.12	392
1988									
27 – 30 June	22	2.9	0.80	0.94***	24 089	3.9	0.64	0.00	97
15–18, 22, 23 August	56	8.8	2.11	0.14	10 275	5.8	0.60	0.02	289
12 – 16 September	68	11.5	2.51	−0.33*	9 270	6.0	0.53	0.13	368
14 – 17, 23 November	10	14.1	2.40	−0.86*	10 992	6.5	0.56	0.41	380
1989									
19 – 22 June	24	3.3	0.85	−0.34	6 869	3.6	0.59	0.32	121
14 – 17, 21, 22 August	237	12.2	2.18	−0.51***	2 525	6.8	0.64	0.02	418
11 – 15 September	188	15.7	2.70	−0.94***	2 542	7.6	0.63	0.25	538
13 – 16, 20 November	10	17.0	3.26	−0.63	2 385	8.1	0.76	0.47	606

[a]Total numbers of age 0 pikeperch caught during one survey were used to estimate standard deviation, skewness, and the significance of the skewness. Those numbers differ from numbers per hour of trawling because total sampling was 2.5 h and geometric means were calculated.

pooled but the smallest size-classes, 4 and 5 cm, could not be analysed due to weight restrictions. All the freeze-dried samples were ground separately and the dry weight of each sample was measured by weighing a subsample of about 2 g, drying it at 105°C for 4 h, and reweighing to a precision of 0.1 mg. The remainder was used to determine the energy content with an adiabatic bomb-calorimeter (IKA-Kalorimeter C400). Both dry weight and energy content measurements were carried out in duplicate. If the energy content of the duplicate determinations differed by more than 2%, the analysis was repeated using new samples. Fat percentages were estimated indirectly by assuming 39.5 kJ·g fat^{-1} and 23.5 kJ·g protein^{-1} (Brafield 1985) and using $\log_e$ ASH = 3.496·$\log_e$ TL − 9.557 where ASH = absolute ash weight (grams) and TL = total length (centimetres) as measured for 2- to 13-cm age 0 pikeperch (n = 49, r^2 = 0.997). Fat content was calculated as

$$\text{Fat} = \left(\frac{\text{EC/AFF} - 23.5}{39.5 - 23.5}\right) \cdot \text{AFF} \cdot \text{DW (\% of FW)}$$

where EC = energy content (kilojoules per gram dry weight), DW = dry weight (percentage of fresh weight), and AFF = ash-free fraction of dry weight, $1 - \text{ASH} \cdot 100 \cdot (\text{FW} \cdot \text{DW})^{-1}$, where ASH = ash weight (grams) and FW = fresh weight (grams).

Results

Log-transformed YCS index and mean length of age 0 pikeperch were positively correlated with water temperature (Fig. 1a and 1b) and with each other (Fig. 1c). The mean length of age 0 fish in the autumn trawl surveys varied between 8.2 and 17.3 cm, while YCS varied between 2.7 and 903 age 0 pikeperch per hour of trawling. Water temperature varied between 327 and 606 degree-days over 14°C. Over the period 1966–89, multiple regression analyses showed that water temperature explained 72% of the variation in length and 35% of the variation in YCS. Growth was highest in the warm summer of 1989, but YCS was much lower than expected.

The length frequency (LF) distributions of age 0 pikeperch during the growing season during 1987–89 illustrated the possible variation in development in size under varying environmental conditions. These three years covered by chance the range in water temperatures observed during long-term recording in Lake IJssel (Fig. 1b and 1c). Both 1987 and 1988 were cold, while 1989 was extremely warm. Growth was slowest in 1987, intermediate in 1988, and fastest in 1989. In August, September, and November 1987, LF distributions were significantly positively skewed (Fig. 2; Table 1). In June 1988, LF distribution was positively skewed, but by mid-August it showed bimodality, although the negative kurtosis, which is an indication for bimodality (Sokal and Rohlf 1981), was not significant (kurtosis = −0.54, $p < 0.2$), with both modes of similar height. In September and November, the importance of the left mode had declined, resulting in a negatively skewed distribution. In 1989, LF distributions were already negatively skewed in August and September.

The abundance, mean length, standard deviation, and skewness in August and November 1966–89 are given in Table 2. Out of 18 years, positively skewed distributions in August were observed in six years and negatively skewed distributions in two years. Skewness (SKNS) was inversely related to mean total length (TL) in August (n = 17, r = 0.63, $p < 0.01$, SKNS = −0.23·TL + 2.45) excluding 1967, when sample sizes were small. For November samples the number of negatively skewed distributions increased to nine out of 24 years.

Food items in the stomachs of age 0 pikeperch were zooplankton, chironomids, *Neomysis integer*, and fish (age 0 smelt or indiscernible). In 1988, the diet of pikeperch larger than 10 cm was exclusively fish (Table 3). A clear difference in the

TABLE 2. Descriptive statistics for age 0 pikeperch in the northern part of Lake IJssel in August and November 1966–89. n = number caught per hour of trawling (geometric mean based on three to eight hauls); TL = mean total length; SD = standard deviation; SKNS = skewness; $*p < 0.05$, $**p < 0.01$, $***p < 0.001$ is the significance of skewness.[a] No data were collected in August in 1974 and 1984–86. Blanks for SD and SKNS indicate that numbers caught were too small for calculations.

	August				November			
	n	TL (cm)	SD (cm)	SKNS	n	TL (cm)	SD (cm)	SKNS
1966	6	9.6	1.20	−0.75	5	14.9	1.40	0.12
1967	2	7.2	1.13	−3.15	11	12.9	2.17	−0.14
1968	1	8.5			5	13.3	2.73	0.84
1969	45	10.3	1.60	−0.21	69	15.5	3.37	−0.41
1970	131	8.5	1.30	−0.20	362	13.7	1.92	−0.68***
1971	151	10.5	1.98	0.42*	30	12.3	2.79	0.18
1972	1	9.0	1.46		23	11.1	2.19	−0.10
1973	149	9.3	1.13	0.42*	212	16.4	2.21	−0.98***
1974					4	13.0	2.88	2.13*
1975	37	10.5	1.47	0.73*	464	16.4	2.00	−0.63***
1976	24	11.6	1.88	0.51	273	17.0	2.05	−0.47***
1977	10	10.3	1.06	0.11	31	11.0	2.38	−0.03
1978	80	7.1	0.89	1.73***	9	8.2	1.20	−0.92*
1979	28	8.7	2.11	0.29	13	13.2	1.35	0.50
1980	11	7.3	1.39	0.95**	15	11.8	2.50	−0.74
1981	44	10.1	1.57	−0.09	31	15.0	1.99	−0.11
1982	115	12.6	1.73	−0.28*	198	17.3	2.12	−0.39**
1983	92	10.1	1.39	−0.09	903	16.7	1.93	−0.53***
1984					16	9.4	1.84	0.93*
1985					47	12.9	2.69	−0.41
1986					64	14.9	1.89	−0.40*
1987[b]	23	6.2	1.21	1.45***	3	12.8	2.79	−1.07*
1988[b]	24	8.5	2.17	−0.12	17	14.4	1.82	−0.45
1989[b]	181	12.6	1.92	−0.54***	13	16.9	3.35	−0.56

[a]Total numbers of age 0 pikeperch caught during one survey were used to estimate standard deviation, skewness, and the significance of the skewness. Those numbers differ from numbers per hour of trawling because the number of hauls and the duration of the hauls varied.

[b]Descriptive statistics for these years differ from those in Table 1. For reasons of comparison with previous years, a smaller number of sampling sites was selected for calculations than in Table 1.

TABLE 3. Number of age 0 pikeperch analysed, percentage of empty stomachs, frequency of occurrence of food items in stomachs as a percentage of fish with full stomachs, mean fresh weight of pikeperch, dry weight (% of fresh weight), energy content (per gram dry weight), and fat weight (% of fresh weight) for different length-classes in the northern part of Lake IJssel, August and September 1988. zoopl = zooplankton; chiro = chironomids; Neomy = *Neomysis integer*.

	Length-class (cm)	Number	Empty (%)	Zoopl (%)	Chiro (%)	Neomy (%)	Fish (%)	Fresh weight (g)	Dry weight (%)	Energy content (kJ)	Fat weight (%)
August	6	40	33	22	—	85	7	1.4	19.6	20.5	0.2
	7	44	36	14	4	68	14	2.2	19.8	20.5	0.5
	8	32	56	—	—	21	79	3.4	19.4	20.6	0.8
	9	13	38	—	—	—	100	5.9	19.7	21.0	0.7
	10	12	58	—	—	—	100	7.9	20.6	21.3	1.3
	11	12	8	—	—	—	100	10.2	21.3	21.6	1.9
	12	8	63	—	—	—	100	13.5	21.6	21.5	1.9
	13	3	33	—	—	—	100	17.1	22.6	21.3	1.7
	14–15	2	50	—	—	—	100	23.6	23.6	21.7	2.4
September	7	25	36	—	—	81	19	2.2	19.0	19.8	−0.1
	8	20	35	—	—	54	54	3.4	19.4	20.0	0.2
	9	19	32	—	—	15	85	5.2	19.6	20.9	1.2
	10	12	42	—	—	—	100	7.9	20.4	21.7	1.8
	11	5	80	—	—	—	100	10.1	21.3	22.1	2.6
	12	6	33	—	—	—	100	13.8	22.0	22.2	2.6
	13	6	50	—	—	—	100	17.5	22.7	22.6	3.4
	14	5	60	—	—	—	100	22.6	23.2	22.6	3.4
	15	3	100	—	—	—	—	28.2	23.9	22.5	3.2
	16–17	3	33	—	—	—	100	38.3	24.0	22.5	3.4

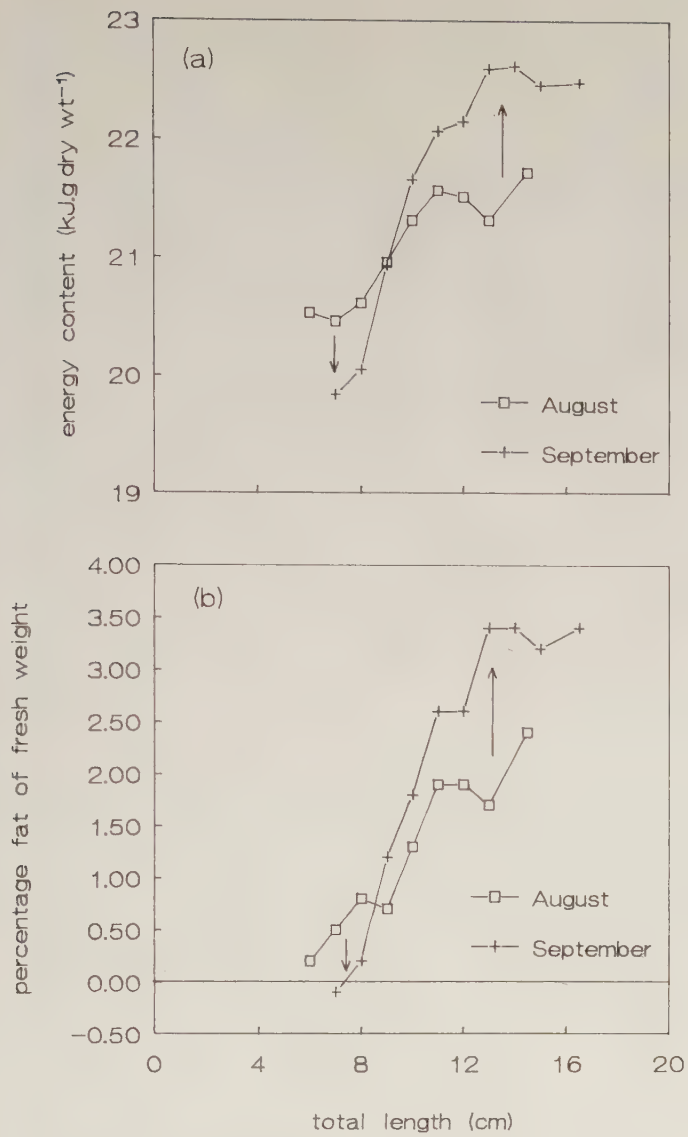

FIG. 3. Energy content and fat weight of age 0 pikeperch in the northern part of Lake IJssel in August and September 1988. Arrows indicate the decrease in condition of small fish and the increase in condition of larger fish.

proportion of piscivores in August between years was observed (Fig. 2). In August 1987, only 11% of pikeperch were piscivorous, while in 1989, almost all (86%) were piscivorous. The situation in August 1988 was intermediate (68%), with the right length mode representing the piscivores. In September 1988 the relative number of piscivorous pikeperch was higher (88%) than in August 1988.

Abundance of age 0 smelt, which was the most important prey fish for age 0 pikeperch, was 10–1000 times higher than age 0 pikeperch in the trawl catches (Table 3). Abundance of age 0 smelt was lowest in 1987, intermediate in 1989, and highest in 1988. Growth of age 0 smelt was slowest in 1988. Degree-days over 14°C were similar in the cooler years 1987 and 1988, but much higher in 1989, which was the warmest summer since the beginning of the survey in 1966. In 1988 the rate of warming was higher at the beginning of the season than in 1987.

The abundance of age 0 pikeperch in June was highest in 1987, but later that year the abundance was lower than in 1988 and 1989 (Table 1). Abundance was high in August and September 1989, which suggested a strong year-class. This was not corroborated by the catches in November. Differences in abundance of age 0 pikeperch in Lake IJssel during 1987–89 were not consistent within years. The catches did not decline over time and thus gave no insight into mortality rates of age 0 pikeperch. The lack of decline in the catches over time was probably due to unknown variation in horizontal distribution patterns and to the effect of varying water clarity on the catchability of pikeperch at the time of sampling.

The mean body weight per length-class was calculated by dividing the total sample weight by the number of pikeperch analysed in that length-class (Table 3). Based on these data the length–weight relationship for age 0 pikeperch was $W = 0.00385 \cdot L^{3.29}$ where W = total weight (grams) and L = total length (centimetres). Total body weight varied between 0.4 g for 4-cm pikeperch and 28.5 g for 15-cm pikeperch in August and between 0.8 g for 5-cm pikeperch and 43.0 g for 17-cm pikeperch in September.

Dry weight, expressed as a percentage of fresh weight, increased with length and varied between 19.0 and 24.0% (Table 3). Dry weight for all length-classes, except for 7-cm pikeperch, was similar in August and September. Energy content per unit dry weight of the total body increased with length (Fig. 3; Table 3). In August, energy content varied 5.7% between 20.5 kJ·g dry wt^{-1} for 6- and 7-cm pikeperch and 21.7 kJ·g dry wt^{-1} for 14- to 15-cm pikeperch. In September, energy content varied 13.2% between 19.8 kJ·g dry wt^{-1} for 7-cm pikeperch and 22.6 kJ·g dry wt^{-1} for 13- and 14-cm pikeperch. The percentage of fat estimated increased from 0–1% for planktivores to 3.5% for piscivores >12 cm in September (Fig. 3; Table 3). The negative value for 7-cm pikeperch in September is probably the consequence of the indirect method of calculation. From August to September, both the energy content and the fat percentage of the total body had declined for pikeperch under over 9 cm and increased for larger pikeperch.

Based on data from van Densen (1985), the ratio between predator size and maximum prey size for age 0 pikeperch and smelt was taken as 1.6. To characterise the availability of smelt for age 0 pikeperch, lengths of smelt were multiplied by this ratio following the method of Shelton et al. (1979). The LF distributions of pikeperch and the availability of smelt are shown in Fig. 4. A clear difference among the years was observed. In 1987, only a small proportion of the smelt was available for pikeperch, so growth of pikeperch was stunted except for a few capable of eating smelt. The converse pattern was found in the warm year of 1989 when almost all pikeperch became piscivorous. An intermediate situation occurred in 1988, and the availability corresponds with the stomach contents (Fig. 1; Table 3).

Discussion

We assume that two processes play a key role in the population dynamics of age 0 pikeperch during summer. First, differential growth between the nonpiscivorous and the piscivorous, and second, size-selective mortality of the smaller nonpiscivorous individuals. Depending on the environmental conditions (water temperature and availability of smelt), both processes can modify the initial LF distribution in a particular way. In a cooler year like 1987, only a few pikeperch were capable of switching to piscivory, which resulted in a stunted population of nonpiscivores with a few larger piscivores, and the LF distribution remained positively skewed. The nonpis-

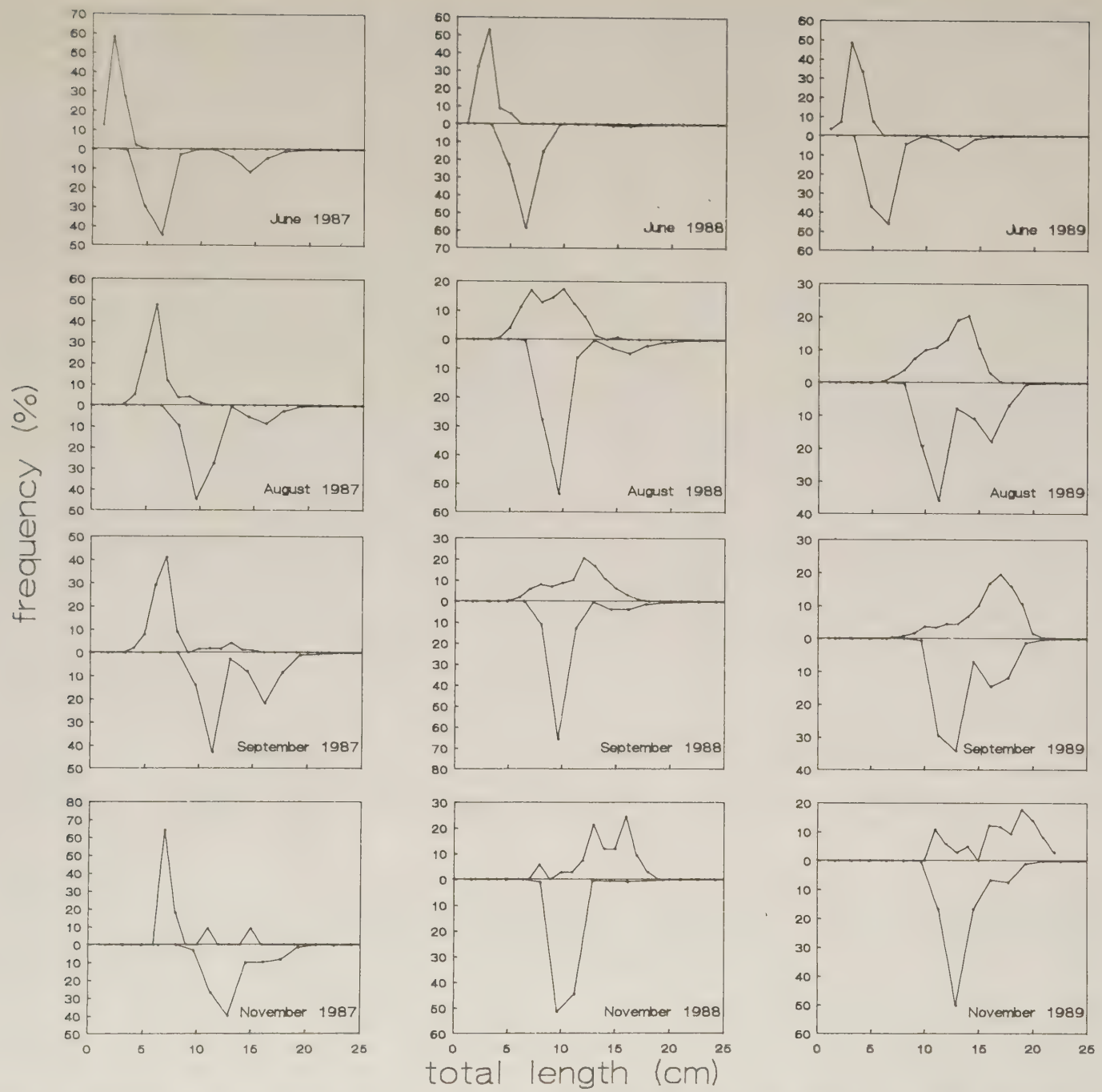

FIG. 4. LF distributions of age 0 pikeperch (above the *x*-axis) and of smelt (below the *x*-axis). Total lengths of smelt were multiplied by 1.6 (predator:maximum prey ratio) to represent smelt available for predation by pikeperch in the northern part of Lake IJssel in June, August, September, and November 1987–89. Smelt that can be preyed upon by a certain size-class of pikeperch are all those straight below and to the left of that size-class.

civores probably have a higher mortality rate, as could be inferred from the diminishing importance of the left mode in the LF distribution in 1988. In the warmer year of 1989, only a few pikeperch were not capable of switching to piscivory, causing a negatively skewed distribution (Table 1).

Theoretically, the declining importance of the left mode in the LF distribution could not only result from size-selective mortality favouring the larger piscivores similar to that found for pikeperch in another Dutch lake (van Densen 1985) and largemouth bass (Shelton et al. 1979; Timmons et al. 1980), but also from accelerated growth of the smaller individuals. The poor condition of the smaller nonpiscivorous individuals, in our opinion, falsifies the hypothesis of accelerated growth. Further, in the period August–September 1987 the increase in modal length of the smaller nonpiscivores was only 1 cm (6–7 cm) whereas in 1989 the increase in modal length of the larger piscivores was 3 cm (14–17 cm). Size-selective mortality is further exhibited by the positive correlation between mean length and abundance of age 0 pikeperch by the end of the first growing season. A notable exception was the year-class 1989, which

displayed the largest growth of pikeperch. Therefore, the strongest year-class was expected. Catches were, however, low in November 1989, which was probably due to unfavourable sampling conditions.

The onset of piscivory is favoured by high temperatures during summer. To be able to ingest smelt, which is the most important food item for age 0 pikeperch in Lake IJssel, a certain ratio must exist (1.6) between prey size and predator size. Since pikeperch profit from high summer temperatures, while smelt do not, a warm summer will result in faster growth of the predator. Total degree-days over 14°C did not differ between 1987 and 1988, but was much higher in 1989, which was reflected in the faster growth of pikeperch. The enhanced growth of age 0 pikeperch in 1988 compared with 1987 was due to more age 0 pikeperch switching towards piscivory in 1988. A warmer spring resulted in a larger mean length of age 0 pikeperch in 1988 by the end of June, and the stunted growth of smelt during the summer of 1988 facilitated the onset of piscivory and rendered smelt more available to predation during the rest of the season. The difference in growth of age 0 smelt between 1987 and 1988 was probably caused by density-dependent processes; the density of age 0 smelt was about six times higher in 1988 than in 1987. Smelt manifest density-dependent growth dictated by availability of zooplankton in other Dutch lakes (van Densen and Vijverberg 1982).

As demonstrated for pikeperch, the onset of piscivory is important for the condition and survival of largemouth bass (Wicker and Johnson 1987). Age 0 largemouth bass in the 10- to 15-cm length groups were emaciated and in poor condition compared with those larger than 17 cm (Adams et al. 1982; Shelton et al. 1979). High mortality periods coincided with diet shifts towards piscivory, indicating that YCS should not be estimated until this bottleneck has been passed (Wicker and Johnson 1987). The explanation given for stunted growth and heavy mortality of largemouth bass is a low ratio of available prey to predator biomass (Timmons et al. 1980; Wicker and Johnson 1987). There is, however, a difference between largemouth bass and pikeperch in timing with respect to their prey. Largemouth bass spawn prior to their prey (Keast 1985; Keast and Eadie 1985). Prey fishes can, however, outgrow the vulnerable size range. Small age 0 largemouth bass shifted from a diet composed of zooplankton, aquatic insects, and larval fish in July to one without fish in September when prey fish became too large to handle, while large age 0 fish remained piscivorous (Keast and Eadie 1985). The importance of early spawning of largemouth bass compared with that of shad (*Dorosoma petenense, D. cepedianum*) for recruitment has also been demonstrated with a predator–prey model by Adams and DeAngelis (1987).

Two theories exist concerning size-selective mortality due to natural causes. The first theory deals with exhaustion through a lack of energy reserves. Winter mortality is size selective because energy reserves are less in smaller contemporaries. Large contemporaries of yellow perch (*Perca flavescens*), (Post and Evans 1989), smallmouth bass (*Micropterus dolomieui*) (Oliver et al. 1979), and largemouth bass (Adams et al. 1982; Aggus and Elliott 1975) survived their first winter better than the smaller ones. The second theory focuses on size-selective survival through predation on the smaller individuals in a cohort (Chevalier 1973; Nielsen 1980).

In this study, size-selective mortality had already occurred during summer and autumn. Invertebrate prey seemed, however, insufficient to sustain the condition of nonpiscivorous pikeperch. Pikeperch of 7 cm, weighing 2.2 g, were analysed both in August and September. Stomachs contained zooplankton and *Neomysis*, and the absolute difference in energy content was $2.2 \cdot (19.8 \cdot 0.190 - 20.5 \cdot 0.196) = -0.616$ kJ. At routine metabolism, the oxygen consumption at 20°C is $0.3 \cdot (2.2)^{0.8}$ $mL \cdot h^{-1}$ (Winberg 1961). For starving fish the energy loss over a 28-d period at 16.8°C would be 5.601 kJ. Consequently the energy loss of 7-cm pikeperch from August to September was 11% of the likely loss after a similar period of starvation. The observed energy contents and fat levels in 6- to 9-cm age 0 pikeperch point, however, towards a poor condition. Yellow perch of 13–18 cm which were starved until mortality occurred contained 2.1% fat based on dry weight (Newsome and Leduc 1975). Age 0 yellow perch in fed and starved treatments suffered 1 and 46% mortality, respectively, mortality being higher among the smaller individuals (Post and Evans 1989). The hypothesis whether planktivorous age 0 pikeperch die of starvation or whether the poor condition of the planktivores results in a higher vulnerability to other causes of mortality such as disease and predation cannot be addressed at this time. Starvation experiments should be conducted to verify whether the smallest specimens in a cohort are able to survive, given the presently observed energy contents per unit dry weight.

Strong year-classes are characterised by a mean length which is larger than average (Willemsen 1977). Our new extended dataset confirmed this and showed a positive correlation between length and survival of age 0 pikeperch. The lower energy content of small nonpiscivorous pikeperch, their declining importance in the LF distribution, and the long-term observation that size and YCS are positively correlated support the assumption that the onset of piscivory in pikeperch populations is an important, and perhaps the most important, YCS-determining process.

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Seasonal Cycle of Zooplankton off Southwestern British Columbia: 1979–89

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Mackas, D. L. 1992. Seasonal cycle of zooplankton off southwestern British Columbia: 1979–89. Can. J. Fish. Aquat. Sci. 49: 903–921.

Average seasonal cycles of zooplankton biomass, species composition, and environmental conditions are estimated from 1979–89 samples collected off the southwest coast of British Columbia. Average total dry weight biomass ranges from a winter minimum <1.5 g/m^2 to a late spring maximum >8 g/m^2. Three subregions within the study area can be distinguished based on bathymetry and current pattern: the inner shelf banks,the shelf break and slope, and a gyre off the mouth of Juan de Fuca Strait. Total zooplankton biomass, species composition, and seasonality differ sharply between subregions, as do temperature, salinity, and nutrient and phytoplankton distributions. From spring through autumn, there are large inputs of upwelled water (and nutrients) to the continental shelf. Phytoplankton biomass is high, especially in mid- to late summer, and is ample to support growth of herbivorous zooplankton. Despite high food availability and relatively low predation pressure, surface layer zooplankton populations decline on the continental shelf from late spring through autumn, while offshore zooplankton populations increase slowly. A high washout rate due to upwelling and subsequent seaward and alongshore transport is the most plausible explanation. For the continental shelf in summer, the advective component of population turnover is probably larger than local predation mortality.

Les auteurs ont estimé les cycles saisonniers moyens de la biomass et de la composition en espèces du zooplancton, ainsi que ceux des conditions environnementales, à partir d'échantillons prélevés de 1979 à 1989 au large de la côte sud-ouest de la Colombie-Britannique. La biomasse totale moyenne (poids sec) variait d'un minimum de $<1{,}5$ g/m^2, en hiver, à un maximum de >8 g/m^2, à la fin du printemps. Les données bathymétriques et les régimes des courants ont permis de délimiter trois sous-régions dans la zone étudiée: la zone interne des bancs, la limite et la pente de la plate-forme et une zone de tourbillon au large de l'embouchure de détroit de Juan de Fuca. La biomasse totale du zooplancton, la composition en espèces et le caractère saisonnier différaient de façon nette entre les sous-régions; il en était de même de la température, de la salinité et des distributions des matières nutritives et du phytoplancton. Du printemps à l'automne, on note d'importants apports d'eau de remontée (et de matières nutritives) sur la plate-forme continentale. La biomasse du phytoplancton est élevée, notamment du milieu à la fin de l'été, et suffit amplement à la croissance du zooplancton herbivore. On note cependant, en dépit de l'abondance de la nourriture et d'une prédation relativement faible, un déclin des populations zooplanctoniques de la couche de surface de la plate-forme continentale, de la fin du printemps jusqu'à l'automne, tandis que les populations du large s'accroissent lentement. Le fort taux de dilution résultant des remontées d'eau et le transport ultérieur vers le large et le long des côtes constituent l'explication la plus plausible de ce phénomène. Quant à la plate-forme continentale en été, la composante advective du renouvellement de la population est sans doute plus importante que la mortalité due à la prédation locale.

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An important goal of biological oceanographic research is to understand how nonbiotic environmental variability affects the composition and total productivity of the plankton and the transfer of planktonic primary and secondary productivity to higher trophic levels. The overall variability of both physical–chemical forcing and biological response includes contributions from a broad spectrum of time and space scales. This has several important consequences. One is that the magnitude of the biological response to a given amplitude of environmental perturbation can be strongly dependent on the time and space scales of the perturbation. A second is that nonlinearities in the interactions among ecosystem components will cause transfer of variability between spectral bands (Steele 1974). A third and very practical consideration is that it can be difficult to quantify the forcing and/or response within one spectral band if there is strong variability in other parts of the spectrum. Detailed and prolonged time-series observations are usually needed to sort out the physical–biological interactions.

In mid- and high-latitude ecosystems, the annual seasonal cycle is usually the strongest environmental signal. But although the absolute range of variation is large, the cycle repeats every year with more or less the same amplitude and phase. Denman et al. (1989) argued that the ability of organisms to deal with environmental fluctuations will vary not only with the frequency and amplitude, but also with the predictability of the fluctuation. Most organisms have developed life history, migratory, and other evolutionarily adaptive strategies that minimize their risk during predictably unfavorable parts of the seasonal cycle. In contrast, environmental anomalies (such as ENSO events) that are aperiodic, and therefore much less predictable, seem able to cause large population-level biological responses despite relatively small absolute magnitudes of the associated abiotic environmental perturbations.

Where seasonality is strong, adequate descriptions of the annual cycles of the biological community and physical environment are an essential first step toward understanding the

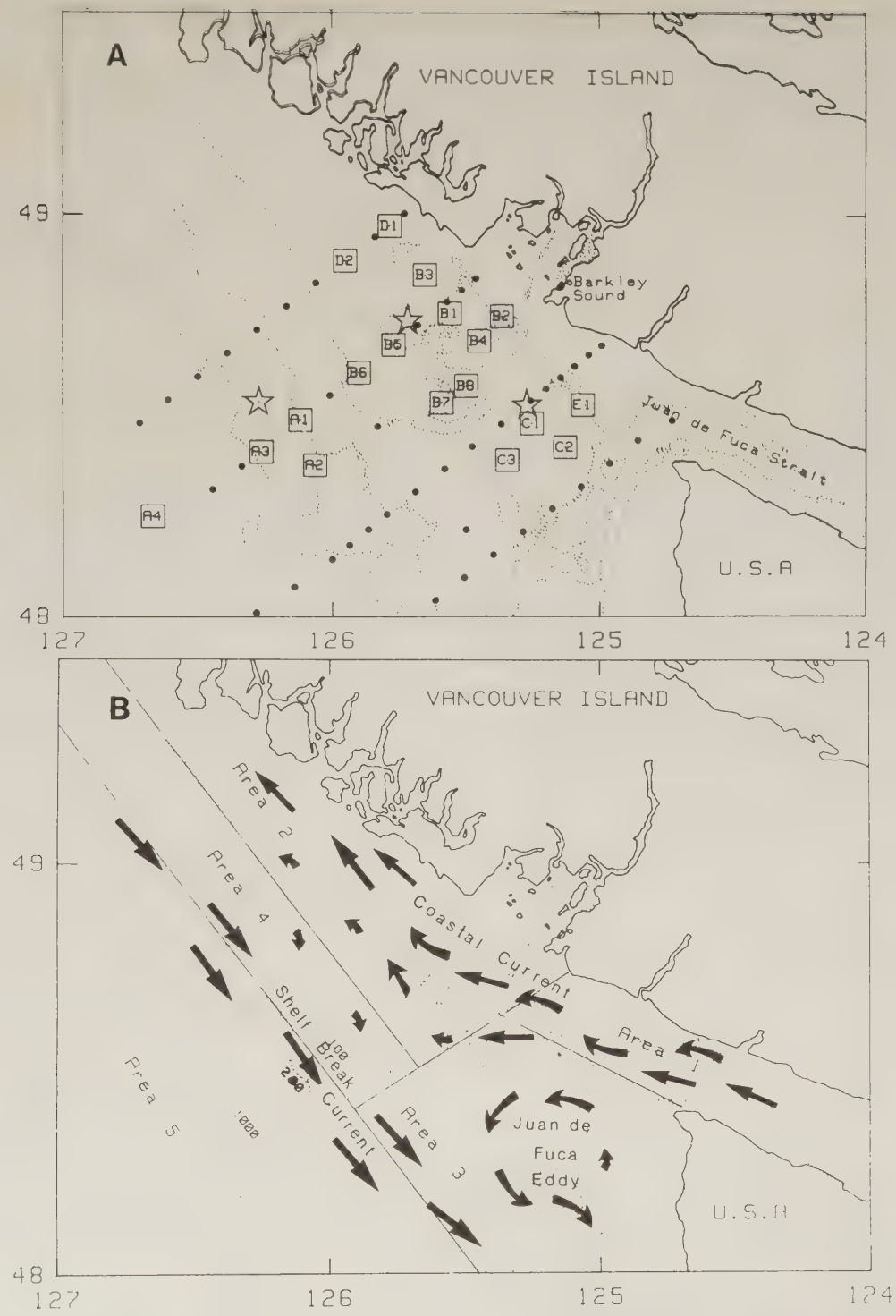

FIG. 1. Sampling locations and statistical averaging regions within the La Perose Project study area off Vancouver Island and their relation to major bathymetric and flow-field features. (A) Standard zooplankton sample sites, 1985–89 (labelled squares), standard CTD sample sites (solid circles), and objective interpolation gridpoints for the temperature and salinity seasonal time series (stars). Zooplankton data were averaged within sample groups B + D + E (inner shelf banks and basins), C (southern shelf gyre region), and A (shelf break and slope). Pre-1985 zooplankton samples from nonstandard locations were assigned to averaging regions based on spatial proximity (see text). (B) Summer upper layer currents (arrows, redrawn from Thomson and Ware 1988), the statistical averaging areas (1–5) used for surface layer nutrient and chlorophyll data, and labels for bathymetric contours.

overall spectrum of variability. In this paper, I estimate the "normal" seasonal cycles of zooplankton biomass and species composition off the southwest coast of British Columbia by averaging data collected over 11 yr (1979–89). Year-to-year deviations from the average pattern will be described in a future paper. Zooplankton annual cycles have been described for neighboring ocean regions off California (Brinton 1976; Colebrook 1977; Chelton et al. 1982) and Oregon (Peterson and

Miller 1977). However, this is the first multiyear analysis for the Pacific coast of Canada (earlier descriptions of temporal variability by Frolander (1962) and Mackas and Sefton (1982) used much shorter time bases; all data from the latter are incorporated in this analysis). Because of strong and relatively persistent mesoscale spatial variability, I also partition the data into three oceanographic regions based on known features of the bathymetry and physical circulation pattern. Finally, I use the seasonally and regionally averaged time series to evaluate the relative importance of zooplankton growth, predation, mixing, and advection rates in setting the overall zooplankton population size.

Overview of Regional Oceanography

The continental shelf and slope waters off Vancouver Island, British Columbia (B.C.) (Fig. 1), are near the northern end of an extensive eastern boundary current/coastal upwelling domain that stretches south to Baja California. The entire system (roughly 25–50°N latitude) is characterized by strong alongshore and cross-shore advective throughput; on the large scale, this is dominated by the average equatorward flow of the California Current and at smaller scales by coastal upwelling circulation and by narrow and intense offshore jets and meanders (e.g. Strub and the CTZ Group 1991). Seasonal variability of large-scale physical processes affecting the continental shelf from California north to the U.S.–Canada border has been described by Strub et al. (1987a, 1987b). More locally, the oceanography of the southern B.C. continental margin has been studied intermittently for 50 yr and regularly and intensively since 1979 (see review in Thomson and Ware 1988).

Even in comparison with other rich midlatitude shelf and eastern boundary current regions, the biological productivity of the southern B.C. shelf is very high. Phytoplankton photosynthesis rates exceeding 20 mg $C \cdot m^{-3} \cdot h^{-1}$ are very common throughout the spring, summer, and early autumn (Forbes et al. 1987; J. R. Forbes, Institute of Ocean Sciences (IOS), pers. comm.). This primary productivity is supported by input of inorganic nutrients via a number of physical processes including alongshore transport from the north, local wind-driven upwelling, interaction of mesoscale currents with bottom topography, tidal mixing, and estuarine circulation in Juan de Fuca Strait (Crawford and Dewey 1989). The annual finfish harvest from the region has varied in this century from 60 to 100×10^3 tonnes (t) fresh weight (roughly 3–5 t/km^2, Thomson and Ware 1988), suggesting that a major fraction of the regional productivity is transferred to higher trophic levels.

Many features of the physical circulation are spatially localized and strongly associated with the coastline and bottom topography. The continental shelf off southern Vancouver Island is wider and topographically more complex than neighboring coastal regions to the north and south (Fig. 1). The southern and widest portion of the shelf is deep (mostly >120 m) and is cut by a submarine canyon system (>250 m) extending seaward from Juan de Fuca Strait. The bottom shoals abruptly off the southeast corner of Barkley Sound forming a series of banks (70–100 m) and semienclosed basins (150–200 m). Further up the coast, the shelf narrows gradually and the outer banks merge with the nearshore shallows. I will distinguish three major subregions (and associated dominant flow-field features) in my discussion of plankton and water property distributions: the shelf break and slope (the region affected in summer by the southeastward flowing Shelf Break Current),

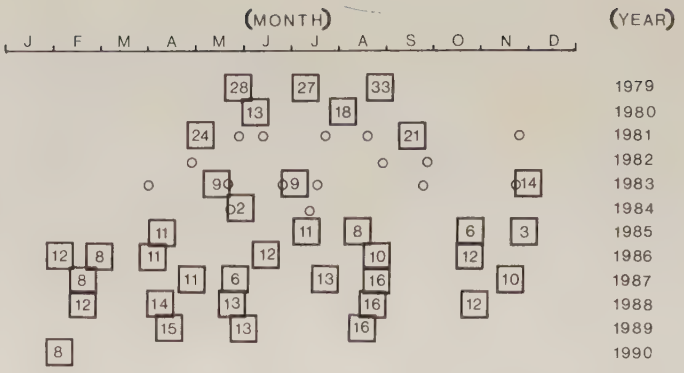

FIG. 2. Time distribution of zooplankton net tow sampling (squares) and supplementary ship-of-opportunity underway sampling (circles) within the study area. Numbers within squares show the number of zooplankton samples collected in each cruise.

the banks and basins of the inner shelf (affected by the northwestward flowing Vancouver Island Coastal Current (VICC), and the deep shelf and submarine canyon region west of the mouth of Juan de Fuca Strait (from spring through autumn the site of a quasi-permanent cyclonic gyre).

At the shelf break, upper layer alongshore currents are strong (often >40 cm/s to depths of about 50 m). The direction of flow is seasonal and is toward the southeast in summer and toward the northwest in winter (Freeland et al. 1984; Thomas and Emery 1986). Changes in current direction accompany the spring and fall transitions in the large-scale weather pattern. Below about 150 m, there is a slower and more persistent flow toward the northwest. Water properties suggest that this is a northward extension of the California Undercurrent (Freeland and Denman 1982; Mackas et al. 1987). Although on average both the Shelf Break Current and the California Undercurrent parallel the shelf break, dynamic instabilities and interactions with local bathymetry generate frequent meanders and mesoscale eddies on the margin of the shelf (Thomson 1984; Ikeda et al. 1984). Especially in summer, upper layer water properties seaward of the Shelf Break Current are relatively "offshore" in character (higher surface temperature, lower dissolved nutrients and phytoplankton biomass). Within the core of the Shelf Break Current, summer levels of dissolved nutrients and phytoplankton biomass are often elevated due to the combined effects of shelf-edge upwelling and alongshore advection (Denman et al. 1981; Simard and Mackas 1989).

The inner and northern part of the shelf is washed over by the low-salinity, buoyancy-driven VICC (Freeland et al. 1984; Thomson et al. 1989). The VICC originates with the estuarine discharge from Juan de Fuca Strait, and receives additional buoyancy input (especially in winter) from local coastal runoff. Surface layer flow is to the northwest throughout the year, but drifter trajectories and maps of dynamic topography show evidence of an alternation between close tracking of the coastline versus an anticyclonic meander extending out over much of the shelf west of Barkley Sound (Thomson and Ware 1988). VICC water is characterized by low salinity. It also often carries very high nutrient loads because of the estuarine circulation and deep tidal mixing at the eastern end of Juan de Fuca Strait (Mackas et al. 1980; Crawford and Dewey 1989).

Summer and autumn circulation at the southeast end of the shelf is frequently dominated by a cyclonic gyre variously called the Tully Eddy or Juan de Fuca Eddy (Freeland and Denman

1982; Freeland et al. 1984; Freeland 1988; Thomson and Ware 1988). The circulation in this southern shelf gyre region appears to be driven by summer season upwelling in the Juan de Fuca submarine canyon system and by the Shelf Break Current (Freeland and McIntosh 1989). Upwelling through the canyon system gives a strong seasonal intrusion of California Undercurrent water and resulting doming of isopycnals and other water property isopleths. Much of the shelf water below about 50 m is flushed each summer by this upwelled California Undercurrent water (Mackas et al. 1987).

Methods

Zooplankton Sampling Schedule, Methods, and Locations

Vertically integrated zooplankton abundance was estimated from the catch of vertical hauls with 0.22-mm-mesh ring (1979–82) or Bongo (1983–89) nets. Tow depths were 250–0 m where bottom depth exceeded 250 m, and near bottom to sea surface elsewhere. Most tows were flowmetered. Where flowmeter readings were unavailable or unreliable, volume filtered was estimated from tow depth and an assumed filtration efficiency of 85%. Formalin-preserved samples were returned to shore for identification and counting to species and size or developmental stage. Sorting effort was stratified by taxa and size class to give reasonably uniform subsampling errors (about ±25–40% within a single sample) for all major groups.

Figure 2 shows timing of zooplankton sampling over the 11-yr period 1979–89. Zooplankton samples were collected during research cruises from two Department of Fisheries and Oceans (DFO) regional laboratories: IOS in Sidney, B.C., and the Pacific Biological Station (PBS) in Nanaimo, B.C. These cruises were supplemented by occasional ship-of-opportunity sampling from DFO research and fisheries patrol vessels. Prior to 1985, the research cruises were "snapshot" studies of the sites and processes responsible for localized regions of high plankton biomass and productivity. Station locations varied substantially from cruise to cruise, and sample timing was heavily biased toward the summer productive season. Previously published analyses of the pre-1985 zooplankton samples (Mackas et al. 1980; Mackas and Sefton 1982; Mackas 1984; Thomas and Emery 1986) have noted strong and relatively consistent zonation of the zooplankton community and biomass patterns. These corresponded closely to the bathymetric and physical circulation features described above.

To provide broader and more consistent spatial and temporal coverage, the two DFO laboratories began in 1985 a long-term collaborative study of factors influencing interannual variability of the continental shelf region off southern Vancouver Island (the La Perouse Project, see Thomson and Ware 1988). Much of the interpretation presented here will be based on 1985–89 data from this project. In contrast with previous efforts, the La Perouse Project zooplankton sampling provided sustained time series at a moderate number of standard locations. These are shown as the small letter-and-number coded boxes in Fig. 1A. The goal was a spatially stratified design in which multiple sample sites could be used to characterize the major oceanographic subregions suggested by earlier studies. The shelf break and slope and the Shelf Break Current are represented by sample sites A1–A4. Sites C1–C3 are from the Juan de Fuca Eddy region. The inner shelf and the Coastal Current are represented by a sequence of three sample groups: E1 just off the mouth of Juan de Fuca Strait, B1–B8 from the banks and basins off Barkley Sound, and D1–D2 from the zone of smoother inner shelf bathymetry extending northwest from the study area. The La Perouse Project grid of stations was sampled four to six times per year from 1985 to 1989 (Fig. 2); coverage averaged slightly over 11 sites per time period and nearly always included two or more samples from each of the major regions.

Sampling of Water Properties, Nutrients, and Phytoplankton

The La Perouse Project zooplankton sampling sites are imbedded in a detailed grid of CTD stations (about 50 sites sampled about 10 times per year, Fig. 1A). Temperature and salinity profiles from these and several earlier surveys have been processed and archived at IOS. I have used summary information extracted from this archive (courtesy R. E. Thomson, IOS) to illustrate the regional seasonal cycles of temperature and salinity experienced by the plankton community.

Water column distributions of dissolved nutrients and phytoplankton biomass were sampled during all of the 1979–84 research cruises and a subset of the 1985–89 La Perouse Project cruises. Collection and analysis methods were as described in Hill et al. (1982). From 1981 through 1984, ships of opportunity also provided data on surface water properties and plankton distributions. Salinity, temperature, chlorophyll fluorescence, and particle abundance were monitored continuously from the ship's seawater supply. Dissolved nutrient, chlorophyll, and preserved phytoplankton and zooplankton samples were collected from the seawater line at roughly hourly intervals. Data from both ships of opportunity (Hoff 1986, unpublished contractor report) and the research cruises were sorted and averaged within the statistical areas outlined in Fig. 1B. The areas were selected to match bathymetric and oceanographic features: (1) Juan de Fuca Strait, (2) the inner continental shelf banks and basins northwest of the mouth of Juan de Fuca Strait (mostly <100 m depth), (3) the southern shelf gyre region (averaging about 150 m depth), (4) the outer continental shelf (mostly 100–200 m depth), and (5) the shelf break and continental slope regions (200–2000 m). The Shelf Break Current flows southeastward roughly along the boundary between areas 4 and 5, while the VICC flows to the northwest through areas 1 and 2, and the cyclonic eddy occupies much of area 3.

Data Reduction, Averaging, and Statistics

From a total list of several hundred zooplankton taxa, I selected a much briefer subset (Table 1) that cumulatively accounted for nearly all (about 90–100%) of the zooplankton biomass. Multiplicative taxon- and stage-specific size coefficients (estimated milligrams dry weight per individual, summarized in Table 1) were used to convert from abundance to "biomass" prior to summation within taxonomic categories. Dry weight coefficients are taken from published (Omori 1969; Harris et al. 1982; Davis 1984; Vidal and Smith 1986; Larson 1986; McLaren et al. 1989) or locally measured fresh-sample dry weight relationships for the most important contributors to total biomass among the taxa in Table 1. Extrapolation from organisms of similar size and shape was used for the remainder. Although bulk dry weight measurements were done on preserved or frozen aliquots from most of the zooplankton samples, these lacked taxonomic information and were also systematically lower by a factor of about 1.5–2 than the within-sample sums of abundance times individual body weight. Bulk

TABLE 1. List of the major zooplankton taxa in continental shelf and slope waters off southwestern B.C., dry weight coefficients used to estimate biomass by taxa, and summary grouping of taxa used in plots of regional seasonal cycles. Body weight ranges are over the range of developmental stages (or body lengths) identified within individual taxa.

Taxon	Body weight (mg DW)	Group classification for plots	
		Major group	Detailed
Euphausiid larvae	0.001–0.04	Euphausiids	(Euphausiid) larval stages
Euphausia pacifica	0.65–8.5	Euphausiids	*E. pacifica*
Thysanoessa inspinata	0.9–10	Euphausiids	*T. inspinata*
Thysanoesssa spinifera	1.25–31	Euphausiids	*T. spinifera*
Parathemisto pacifica	0.75–3.7	Amphipods	n.a.
Primno	0.32–40	Amphipods	n.a.
Acartia longiremis	0.002–0.007	Calanoid copepods	*Acartia*
Acartia clausi	0.002–0.007	Calanoid copepods	*Acartia*
Calanus marshallae	0.008–0.23	Calanoid copepods	*C. marshallae*
Pseudocalanus	0.0018–0.012	Calanoid copepods	*Pseudocalanus*
Oithona similis	0.0005–0.001	Cyclopoid copepods	*O. similis*
Metridia pacifica	0.012–0.14	Calanoid copepods	*M. pacifica*
Neocalanus cristatus	0.04–2.6	Calanoid copepods	Subarctic (copepods)
Neocalanus plumchrus	0.013–0.55	Calanoid copepods	Subarctic (copepods)
Eucalanus bungii	0.008–0.68	Calanoid copepods	Subarctic (copepods)
Euchaeta elongata	0.035–1.6	Calanoid copepods	Subarctic (copepods)
Racovitzanus antarcticus	0.016–0.1	Calanoid copepods	Subarctic (copepods)
Mesocalanus tenuicornis	0.007–0.4	Calanoid copepods	Southern (copepods)
Clausocalanus	0.0025–0.008	Calanoid copepods	Southern (copepods)
Ctenocalanus vanus	0.004–0.012	Calanoid copepods	Southern (copepods)
Eucalanus californicus	0.025–1.3	Calanoid copepods	Southern (copepods)
Acartia danae	0.0015–0.005	Calanoid copepods	Southern (copepods)
Aetideus divergens	0.023–0.05	Calanoid copepods	Other calanoids
Calanus pacificus	0.003–0.22	Calanoid copepods	Other calanoids
Calocalanus	0.0005–0.003	Calanoid copepods	Other calanoids
Centropages abdominalis	0.005–0.03	Calanoid copepods	Other calanoids
Microcalanus pygmaeus	0.0015–0.0035	Calanoid copepods	Other calanoids
Paracalanus parvus	0.0015–0.0065	Calanoid copepods	Other calanoids
Scolecithricella minor	0.0035–0.011	Calanoid copepods	Other calanoids
Oithona atlantica	0.002–0.0035	Cyclopoid copepods	Other cyclopoids
Corycaeus	0.001–0.005	Cyclopoid copepods	Other cyclopoids
Oncaea	0.002–0.004	Cyclopoid copepods	Other cyclopoids
Juvenile chaetognaths	0.014–0.063	Chaetognaths	(Chaetognath) juveniles
Sagitta elegans	0.1–1.0	Chaetognaths	*S. elegans*
Sagitta scrippsae	0.18–6.5	Chaetognaths	*S. scrippsae*
Eukrohnia hamata	0.18–1.4	Chaetognaths	*E. hamata*
Limacina	0.1–1	Pteropods	n.a.
Larvaceans	0.01–0.2	Urochordates	Larvaceans
Salps	7–130	Urochordates	Salps
Doliolids	0.2–1.2	Urochordates	Doliolids
Siphonophores	0.2–15	Cnidaria	Siphonophores
Hydromedusae	0.5–60.0	Cnidaria	Hydromedusae
Scyphomedusae	200	Cnidaria	n.a.
Ctenophores	0.9–6.9	Ctenophores	Ctenophores
Cladocera	0.005	Remainder	n.a.
Conchoecia	0.1	Remainder	n.a.
Barnacle larvae	0.04	Remainder	n.a.
Eggs	0.0016	Remainder	n.a.
Clione	0.5–3.5	Pteropods	n.a.
Mollusc larvae	0.002	Remainder	n.a.
Tomopteris	0.04–0.3.5	Remainder	n.a.

dry weight mesurements are liable to a downward bias of about this magnitude from leakage and shrinkage losses (Giguère et al. 1989). I therefore view the taxonomically partitioned "biomass" estimates calculated from the abundance data as substantially more informative and somewhat more reliable than the bulk measurements.

I computed within-survey community resemblance (as chord length distance, Orloci 1978) for each of the 1985–89 La Perouse Project cruises using the full list of taxa in Table 1 (earlier data were analyzed similarly by Mackas and Sefton 1982). I then used cluster analysis by average linkage and sum-of-squares algorithms (Orloci 1978; Mackas and Sefton 1982) to group samples with similar species composition ratios. Comparison of the "objective" groupings of samples by the cluster analyses (based on species composition) with the prior spatial classification of La Perouse sample sites (based on bathymetry

and current patterns) provided a check on the validity of within-region averaging and a basis for possible adjustment of sample site classification.

Finally, I transferred the merged and size-weighted zooplankton data matrix (samples by taxa) to spreadsheet format for sorting, averaging, and plotting of community composition within time periods and spatial regions. To improve clarity of these plots, I have further simplified the list of taxa by additional merging steps (summarized in Table 1). Five sets of plots will be presented: (1) a "major groups" summation of all taxa, plus more detailed breakdowns of composition within the (2) copepod, (3) euphausiid, (4) chaetognath, and (5) gelatinous zooplankton subgroups.

To focus on the annual cycle within each oceanographic subregion, I averaged spatially within regions and averaged seasonally between years. Because the most important goal of this paper is to provide an unbiased estimate of the seasonal cycle of composition and biomass in each region, rather than to test rigorously for statistical significance of the relatively large differences between regions and time periods, I have used arithmetic averages of untransformed zooplankton data throughout. This has both advantages and disadvantages (Colebrook 1977). Arithmetic averages provide unbiased estimates of total population sizes. But because the statistical distribution of individual sample estimates is strongly skewed, the averages converge slowly toward normal distributions and tend to be unstable because they are strongly influenced by the small number of samples with very high zooplankton densities.

The averaging of samples proceeded in two steps. The first step was within-cruise spatial averaging into "shelf break and slope," "southern shelf gyre," and "inner shelf banks and basins" regions. For the La Perouse Project cruises (1985–89), these spatial averaging units correspond to the "A," "C," and "B + D + E" sample groups, respectively (Fig. 1A). For the earlier cruises (1979–84), samples located in the ship-of-opportunity statistical areas 1 and 2 and the inshore half of 4 were taken as representative of the inner shelf, those from statistical area 3 the gyre region, and samples from area 5 and the outer half of 4 the shelf break and slope (see Fig. 1B).

The second averaging step was time averaging of the within-cruise spatial means to produce a composite zooplankton seasonal cycle for each of the three major regions. I partitioned the annual cycle into six segments (February, April, May–June, July, August–September, and October–November) that gave both approximately equal time spacing and a similar number of cruises in each time block. The number of samples per region varied between cruises (as few as 1 and as many as 22). The relative weighting of within-survey within-region means therefore determined how strongly each sample (or each survey) contributed to the overall within-season time average. Because of nonnormality of the underlying distributions and heterogeneity of variance across taxa, regions, and time periods, an objectively "best" weighting scheme was not obvious. I used weighting proportional to the square root of the number of within-region samples in each cruise (proportional to $(N_i)^{0.5}$ where N_i is the number of individual samples used to form the within-survey mean in cruise i). This choice is intermediate between the two simplest alternatives: (1) uniform weighting of within-cruise means (proportional to $(N_i)^0$; appropriate if within-region spatial patchiness is insignificant compared with the variance contributed by interannual variability) and (2) uniform weighting of individual samples (proportional to $(N_i)^1$; appropriate if year-to-year differences are small and small-scale patchiness is intense). Because both small-scale patchiness and interannual differences are significant, weighting proportional to $(N_i)^{0.5}$ provides a reasonable intermediate. However, for comparison purposes, I also calculated seasonal cycles using schemes (1) and (2) and will show later that there was remarkably little difference between the zooplankton seasonal cycles as estimated by the three schemes.

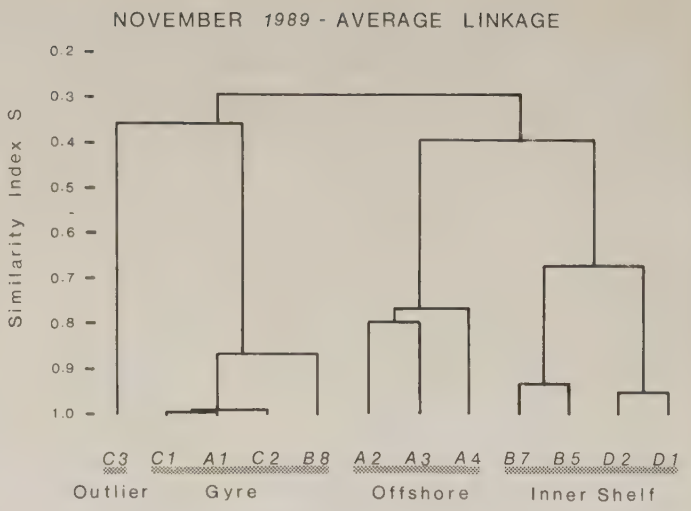

FIG. 3. Dendrogram showing the grouping of November 1989 zooplankton samples based on resemblance of zooplankton community composition. Individual sample labels are site codes from Fig. 1A. Regional labels assigned to the sample groups are shown at the base of the dendrogram. Although the dominant species within each region shifted with season, there was a strong and consistent tendency for neighboring samples to cluster together and for cluster spatial boundaries to coincide with transitions in bathymetry and current direction.

For a description of seasonality of the physical environment, the CTD data archive from the La Perouse Project provides the best available time- and space-resolved information on water properties. One of the standard analyses applied to these data is a cruise-by-cruise objective interpolation of the measurements to a coarse three-dimensional grid (20 km horizontal spacing, 0, 10, 20, 50, and 100 m depths). The assumed correlation length scales for this interpolation are 50 km alongshore and 25 km cross-shore. Seasonal time series were then derived from the objective maps by sorting the calculated gridpoint estimates into bimonthly time bins and averaging across years (data and analysis courtesy R. E. Thomson, IOS). I have extracted from the overall archive the three gridpoints located closest (see Fig. 1A) to the centers of the three La Perouse zooplankton sample clusters and generated season versus depth contour plots of temperature and salinity.

Seasonal cycles of surface layer dissolved nutrients and phytoplankton biomass were estimated by space and time averaging of data from both the ships of opportunity (nominal 3 m sampling depth) and 1979–89 research cruises (all water samples 0–10 m). Within-cruise spatial averaging was arithmetic and used the ship-of-opportunity statistical areas (Fig. 1B). The cruise regional means were then sorted and averaged into six approximately bimonthly time categories. Time averaging was arithmetic for nitrate and geometric for chlorophyll concentration. The latter procedure gives a downward bias of the within-season mean but, by giving symmetric confidence intervals, allows a more realistic impression of cruise-to-cruise within-season variability.

Results and Discussion

Cluster Analysis of La Perouse Sampling Sites

I will first consider whether the prior spatial classification of La Perouse Project sampling sites provides a reasonable basis for within-time-period averaging. I do this by comparing the spatial groupings with the results of within-time-period cluster analysis (e.g. the dendrogram of November 1989 samples shown in Fig. 3). The cluster analysis produces a classification based on resemblance of species composition and therefore suggests the borders of community composition "patches" for the particular time period being analyzed. The actual composition within the patch will vary with season; the question is whether the location of patch boundaries is both reasonably stable over time and congruent with the prior, fixed classification of sites by position, current pattern, and bathymetry. If the answer is yes, the spatial groups represent natural averaging units; if no, the averaging units could be merged or otherwise redefined.

Cluster analyses usually classified the sites into three or four (occasionally two) groups indicated along the bottom margin of the dendrogram in Fig. 3. For comparison across time periods, I then assigned labels to the same clusters as follows: "offshore" (including offshore end-member sites A4 and/or A3), "inner shelf" (including sites B6, B7, and/or B5), "gyre" (any clear clustering of C region sites that did not fall within one of the two preceding groups), and "outlier" (none of the above). Both internal resemblance and strength of linkage to the other regions usually were lowest for the "offshore" group.

Figure 4 shows the frequency with which each La Perouse site was assigned to each of the above classes. Overall cluster membership corresponded well with the initial spatial groupings of sites, confirming their suitability as averaging units. A few sites (notably A1, B8, and C3) showed frequent crossovers to adjoining regions; these are also the "borderline" sites in position and depth range (see Fig. 1). An interesting result is the consistent species-based classification of site E1 (at the mouth of Juan de Fuca Strait) as a member of the "inner shelf" cluster. This supports the hypothesis that the inner shelf zooplankton community is very heavily influenced by the VICC (for which the upstream source is the estuarine discharge from Juan de Fuca Strait).

Seasonal Cycles of Temperature and Salinity

Figure 5 shows the estimated seasonal cycles of temperature and salinity in the upper 100 m at objective map gridpoints within each of the three La Perouse zooplankton averaging regions (locations shown in Fig. 1A). The major between-region difference in mean water properties is the lower salinity in the inner shelf region. This again reflects the strong regional influence of the VICC.

Seasonal variability is apparent in all three regions. The two most obvious seasonal signals are summer warming of the water above about 50 m and significant cooling of the deeper layers due to the strong summertime intrusion of cold, high-salinity upwelled water. The three regions differ in the amplitude and depth distribution of their seasonal variability, as indicated by the number of contour line crossings at a given depth between annual maxima and minima. The surface layer warming is strongest in the outer shelf region and weakest in the southern shelf gyre region. Conversely, the upwelling signal at 100 m is strongest in the gyre region and weakest offshore.

Although the effects of the summer season upwelling are most obvious and unambiguous below 50 m, there is also a major influence on upper layer water properties. The lowest surface salinity occurs in spring in all three regions. This timing is not attributable to the seasonality of coastal freshwater input, since it occurs during a seasonal minimum in total precipitation and runoff (Freeland et al. 1984). Changes in salinity correlate better with the timing of the seasonal upwelling (note in the inner and southern shelf profiles the synchrony between the late spring increase of salinity in the surface layer and the upward movement of isotherms and isohalines at depth), and in turn with the large-scale seasonality of alongshore winds and currents. The spring transition to upwelling-favorable winds occurs

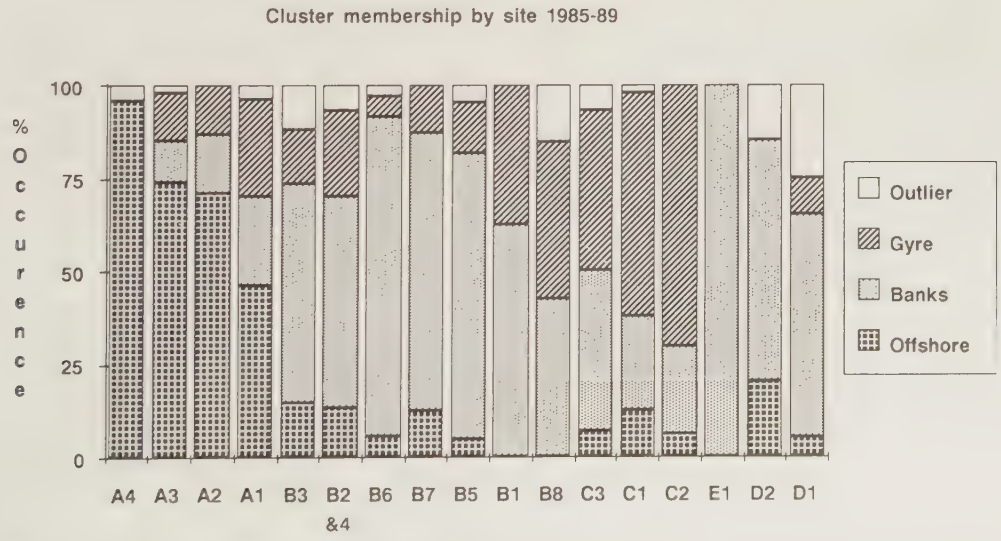

FIG. 4. Relative frequency (over all sampling periods) at which each of the standard La Perouse zooplankton sampling sites was grouped by cluster analysis into identifiable oceanographic regions. The cluster analysis classification (based on zooplankton species composition within samples) agrees closely with the prior regional labelling of samples (based on location and bathymetry). This supports the use of the regional groups as statistical averaging units.

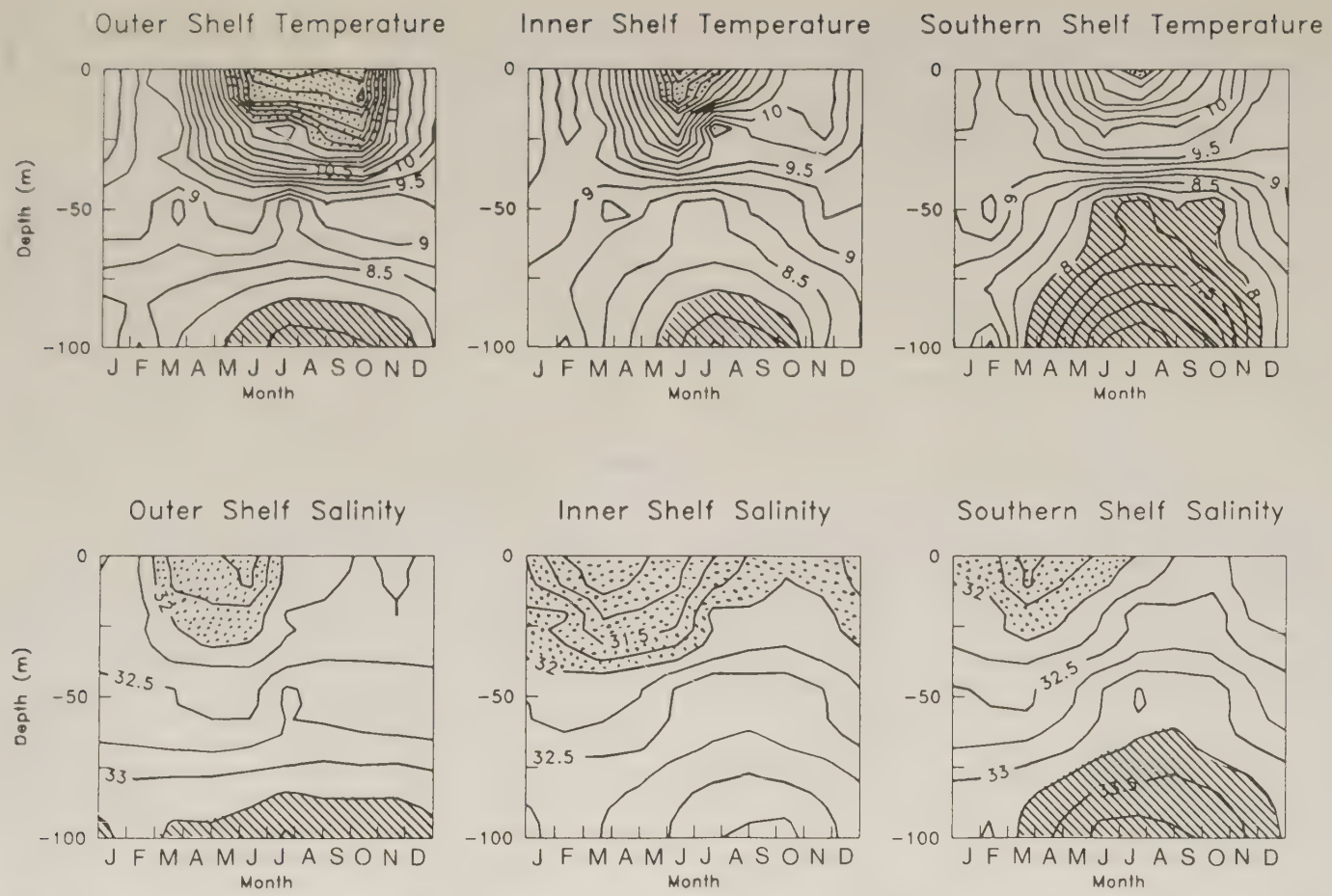

FIG. 5. Average seasonal cycles of temperature and salinity versus depth estimated (by objective interpolation from adjoining CTD sample sites) for locations representative of each of the three zooplankton averaging regions (Fig. 1A). Stippled areas indicate temperatures warmer than 11.5°C and salinities fresher than 32 PSU. Diagonally hatched areas indicate temperatures colder than 8°C and salinities higher than 33.25 PSU. The major seasonal signals are the spring and summer warming of the surface layer and the intrusion at depth of cold saline upwelled water (strongest in the southern shelf gyre region). Because of the influence of the VICC, the inner shelf region has relatively low surface layer salinity year-round. However, the summer increase in salinity and decrease in temperature indicate admixture of upwelled water.

in late March to early April (Freeland et al. 1984; Strub et al. 1987a). Prior to this, coastal freshwater inputs are retained on and near the shelf. After the spring transition and continuing through to the fall transition in September–October, shelf surface waters experience net seaward Ekman transport, plus dilution by proximity to and mixing with more saline upwelled deep water. Much of the transfer of upwelled water into the surface layer of the inner shelf probably occurs via a circuitous route: deep inflow of California Undercurrent water up the Juan de Fuca Strait and submarine canyon system, strong tidal mixing near the inner end of the strait, and eventual transport back out to the shelf in the surface layer estuarine discharge (Mackas et al. 1980, 1987; Crawford and Dewey 1989).

Note also the between-region differences in phasing of the seasonal temperature maxima and salinity minima (inner shelf earliest, outer shelf latest). This is also consistent with a seaward propagation of upwelled water, superimposed on the much stronger alongshore flow of the shelf break and coastal currents.

Seasonal and Regional Variability of Dissolved Nutrients and Phytoplankton Biomass

Seasonality by region of surface layer dissolved nutrient and phytoplankton concentrations is shown in Fig. 6. During the late spring and summer seasons, there is intense variability at short time and space scales in all regions because of localized and episodic phytoplankton blooms (note within-time-period range of means in Fig. 6). Individual bloom and decline cycles are not resolved because of the time gaps between sampling periods and the 1- to 2-mo averaging interval.

Several features should be noted. The first is the overall high summer season concentrations of both nitrate and chlorophyll. The second is their general cross-shelf gradient. On the inner part of the shelf (regions 1–3), average surface layer nitrate concentrations >10 μM and chlorophyll concentrations >5 mg/m^3 are common. The offshore levels (regions 4 and 5) are usually lower (about 1–5 μM nitrate and 1–3 mg chlorophyll/m^3 but remain large when compared with other midlatitude oceanic regions.

The third important point is the unusual timing of the seasonal maxima and minima. The lowest surface nutrient concentrations are in March–April in Juan de Fuca Strait and May–June in all other regions; nitrate concentrations increase throughout the summer and reach their annual maximum in August–September (Juan de Fuca) or October–November (other regions). High average chlorophyll concentrations occur throughout the spring, summer, and early autumn on the inner shelf. In the two offshore regions, phytoplankton biomass

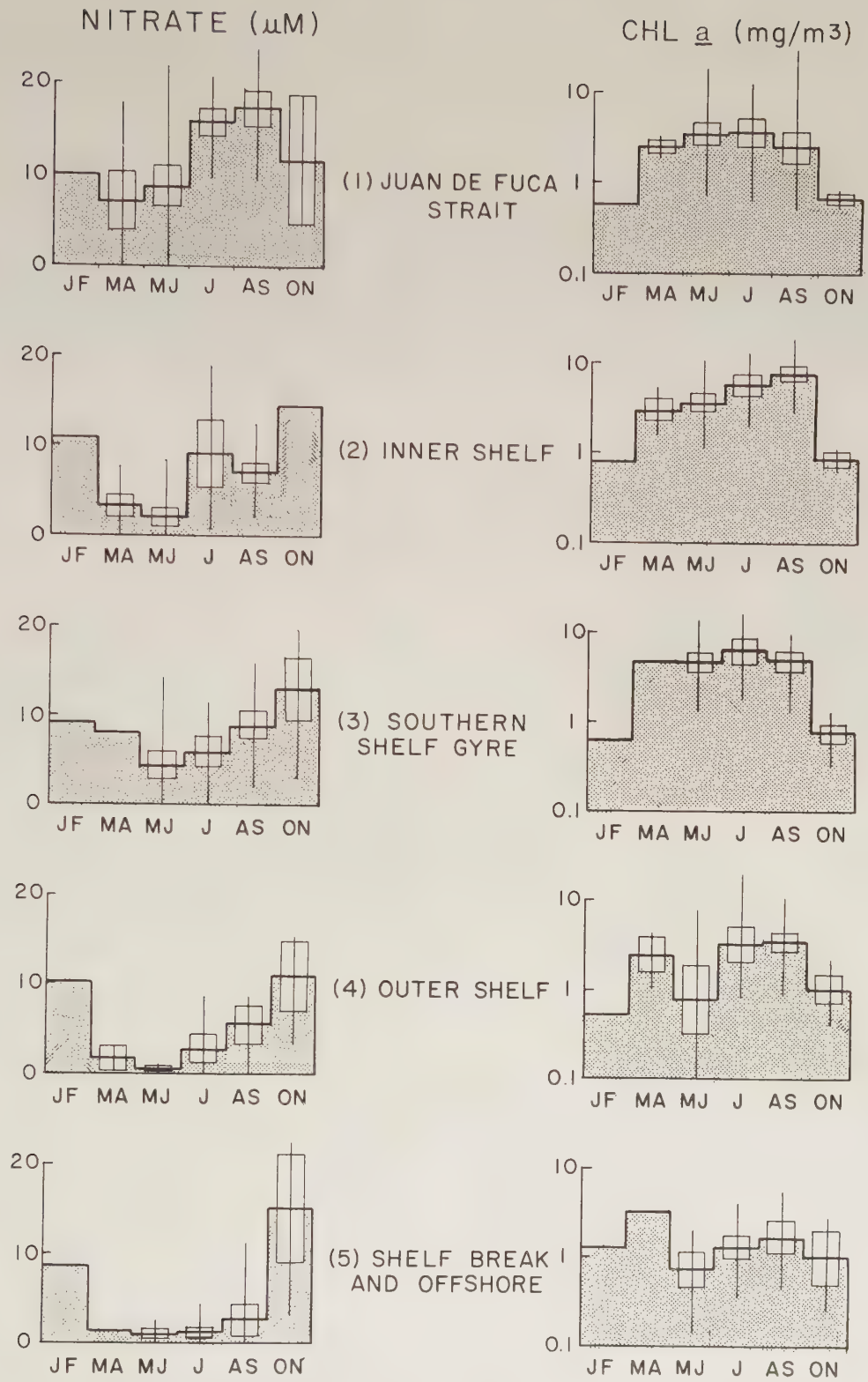

FIG. 6. Seasonal cycles of surface layer (0–10 m) nutrients (as dissolved nitrate) and phytoplankton biomass (as chlorophyll *a* concentration, logarithmic scale) for the statistical averaging areas shown in Fig. 1B. The shaded area shows the multiyear average within bimonthly time intervals; where data are available from more than one cruise per time bin, boxes and lines indicate, respectively, the standard error and total range of within-cruise within-region spatial averages. Although there is a strong cross-shelf gradient (top panels of the figure show nearshore regions, bottom panels offshore), average spring and summer season nutrient and phytoplankton concentrations are relatively high in all regions. Surface layer nitrate is very rarely depleted to undetectable levels, suggesting a high percentage of exportable "new" primary and secondary production. Note also the unusual seasonal cycle with maximum phytoplankton biomass occurring in mid- to late summer in most regions. This further supports the interpretation of strong midsummer inputs of nutrient-rich upwelled water.

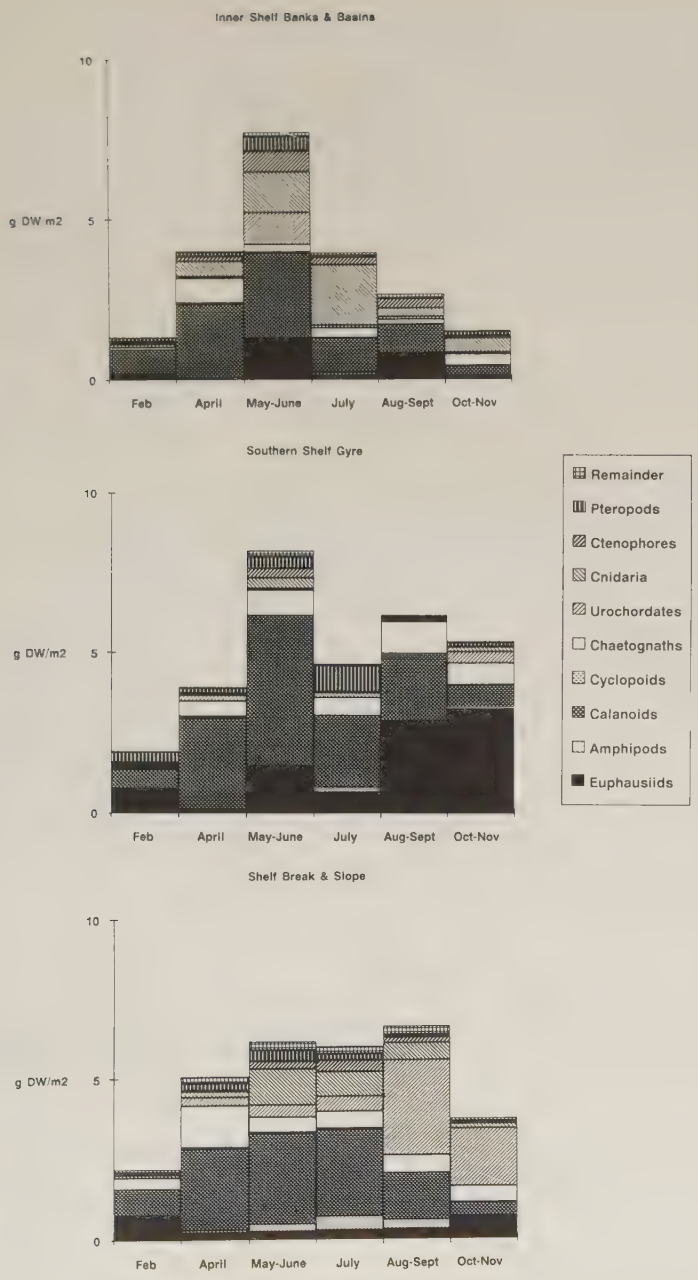

FIG. 7. Average seasonal cycles off the outer coast of Vancouver Island of total zooplankton biomass subdivided by major taxonomic group (biomass as estimated grams dry weight (DW) per square metre). From top to bottom, the three zooplankton spatial averaging regions (see text) are inner shelf banks and basins (sites labelled B, D, and E in Fig. 1A and 4), southern shelf "gyre" (sites labelled C), and the offshore shelf break and slope (sites labelled A). Zooplankton species making up the taxonomic groups shown here are listed in Table 1 (Fig. 9–12 show more detailed seasonal breakdowns within the copepod, chaetognath, euphausiid, and gelatinous zooplankton categories). The dominant taxa are calanoid copepods and jellyfish on the inner shelf banks, calanoids and euphausiids in the (deeper) southern shelf gyre region, and calanoids, euphausiids, chaetognaths, and salps along and seaward of the shelf break. The seasonal peak zooplankton biomass (mostly made up of herbivores) occurs in late spring and very early summer on the continental shelf; the subsequent midsummer decline is during a period of sustained high availability of phytoplankton and is therefore unlikely to be due to food limitation. In contrast, offshore zooplankton populations show a gradual but sustained seasonal increase through to the end of summer, despite much lower average levels of phytoplankton food.

decreases in May–June following partial depletion of nutrients by spring bloom(s) in March–April. But the chlorophyll levels do not remain low. Instead, they increase in mid- and late summer along with the upper layer nitrate concentration. On average, phytoplankton populations experience high to very high availability of dissolved "new" nutrients and are capable of high productivity in all but the light-limited winter season. Like the temperature and salinity distributions discussed above, the nitrate and chlorophyll data show strong and persistent (or perhaps recurrent) summer season input of upwelled water. Regional differences in level and phasing of the nitrate cycle (highest and earliest in Juan de Fuca Strait) confirm the interpretation (Mackas et al. 1980; Crawford and Dewey 1989) that the estuarine circulation in the strait is a principal intermediate pathway for this input.

Zooplankton Composition and Biomass Cycle

Overview of major taxonomic groups

The estimated "average" seasonal cycles of total zooplankton biomass and the relative contribution of the coarse taxonomic groups to this total are shown in Fig. 7. All regions share the expected winter minimum and spring–summer maximum of zooplankton biomass. Superimposed on this are large between-region differences in zooplankton species mix and in amplitude and phase of the seasonal cycle. How sensitive are these patterns and their differences to variability among the samples being averaged and to my choice of sample weighting scheme? The values plotted in Fig. 7 were calculated using the "intermediate" ($(N_i)^{0.5}$) weighting scheme. Figure 8 shows that the choice of sample weighting scheme had very little effect on the estimated seasonal pattern in the offshore region; the inner shelf and gyre regions (not shown) had even stronger between-scheme resemblance.

There are important differences in the stability and reliability of seasonal averages between the various taxa shown in Fig. 7. Several sources of variability contribute to within-averaging-bin variance: total within-sample abundance, size resolution for abundance to biomass conversion coefficients, intensity of small-scale spatial patchiness, and intensity of interannual variability. These differ between taxa in their absolute and relative importance (to be discussed in subsequent sections on individual taxonomic groups). But in general, the seasonal and regional averages are most reliable for small and abundant organisms such as copepods, larvaceans, and euphausiid larvae; they are least reliable for large and numerically rarer taxa such as adult euphausiids, salps, and medusae.

Of the three regions shown in Fig. 7, the inner shelf consistently had the lowest average biomass (annual average 3.2 g/m^2), the narrowest seasonal peak (averaging about 7.5 g/m^2 for a brief period in late spring to early summer), and the lowest winter levels (1–1.5 g/m^2). Calanoid copepods and/or jellyfish are the dominant taxa in all time periods. Compared with the other regions, chaetognaths and euphausiids made small contributions to the total. This in part reflects exclusion because of bottom depths shallower than their normal diel migration range. The biomass peak on the inner shelf occurs early in the productive season (May–June) and coincides with the timing of the upper layer temperature maximum (Fig. 5) and dissolved nutrient minimum (Fig. 6). The subsequent mid- to late summer drop in total and herbivorous zooplankton biomass contrasts with the increasing levels of dissolved nutrients and phytoplankton biomass shown in Fig. 6.

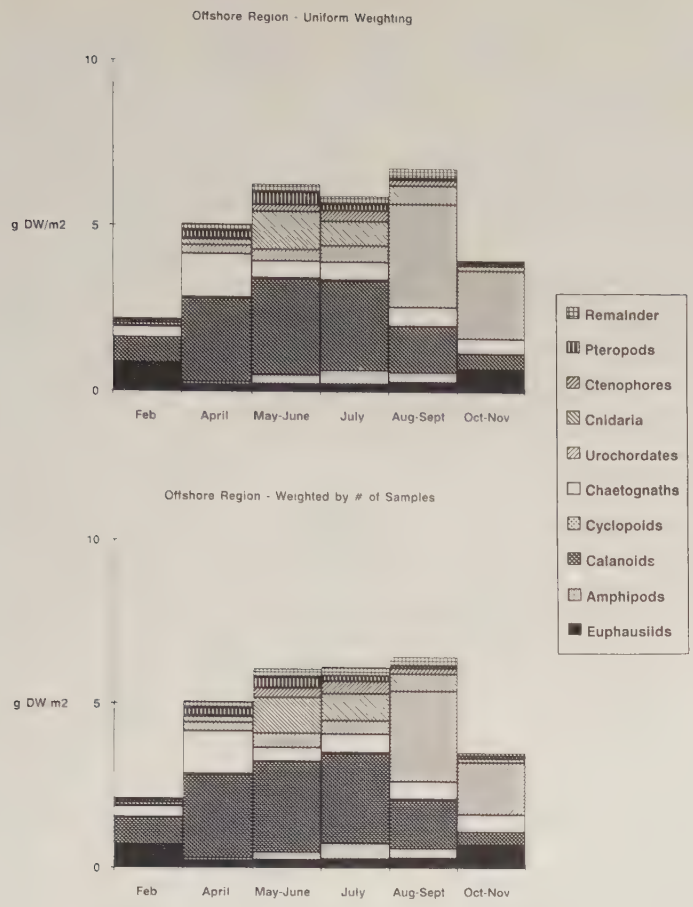

FIG. 8. Stability of the estimated zooplankton seasonal cycle in the offshore (shelf break and slope) region despite changes in the sample weighting scheme used to calculate the seasonal averages. For the upper panel, the weighting scheme (designed to filter out between-year temporal variation) is uniform for each time period (= cruise) sampled and therefore independent of the number of samples (N = 1–22) forming the within-cruise means. For the lower panel, the weighting scheme (designed to filter out within region spatial patchiness) is uniform for each individual location sampled and therefore linearly proportional to the number of samples forming each within-cruise mean. Results are insensitive to the weighting scheme used. Compare also Fig. 7, which was calculated using (along with Fig. 9–12) a standardized "intermediate" weighting scheme proportional to $N^{0.5}$.

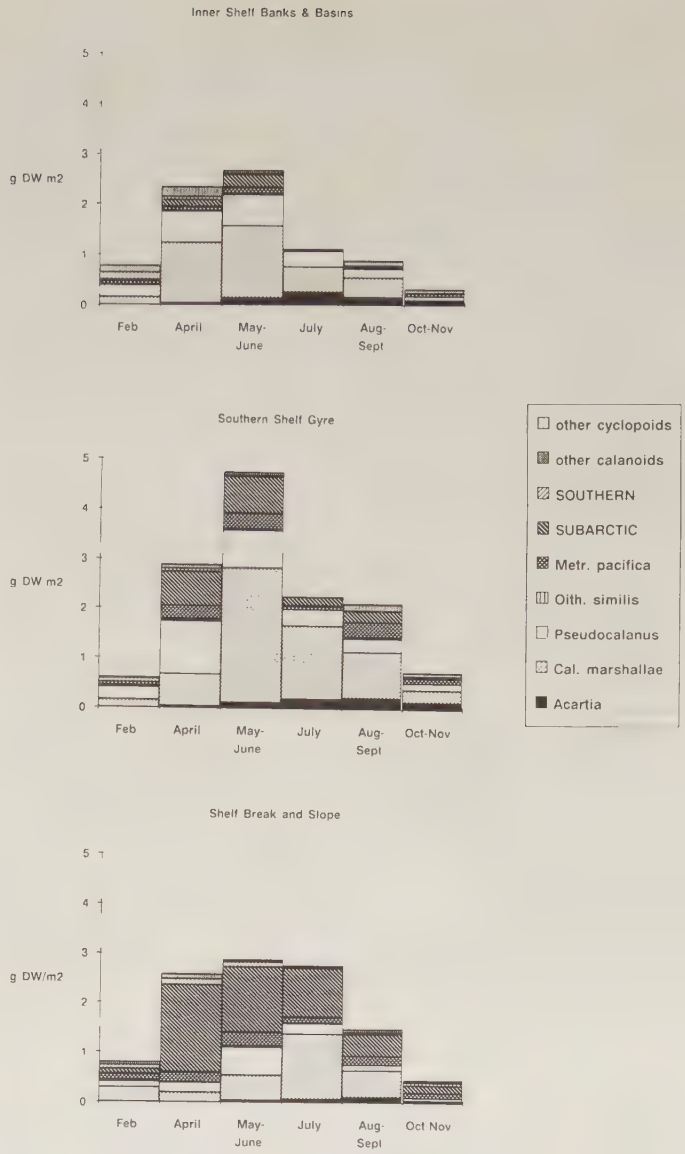

FIG. 9. Average seasonal cycles of copepod biomass off the outer coast of Vancouver Island. Spatial averaging is as in Fig. 7. The dominant taxa on the shelf are the calanoids *Calanus marshallae* and *Pseudocalanus* spp. (mostly *P. mimus*). Offshore, there is also a high spring and early summer biomass of copepods endemic to the open subarctic Pacific (mostly *Neocalanus cristatus* and *N. plumchrus*). As with total zooplankton biomass, the shelf regions have a strong seasonal maximum in spring followed by a summer decline. The offshore region has a more delayed and much more sustained summer maximum; *Pseudocalanus* and *C. marshallae* increase in abundance offshore at the same time as they are in decline on the shelf, suggesting seaward transport of shelf zooplankton.

Biomass in the southern shelf gyre region (annual average 4.9 g/m^2) was much greater than the inner shelf and slightly higher than the shelf break and slope. Like the inner shelf, the gyre region had peak biomass (>8 g/m^2) in late spring to early summer, but the subsequent dropoff from this peak was weaker and less sustained than on the inner shelf. The summer decrease in the biomass of calanoid copepods from their May–June maximum was partially offset by a late summer increase in the average biomass of euphausiids. However, the high euphausiid biomass in the final third of the year was due mostly to a small number of samples from 1988 and 1989 and may therefore not be a stable feature of the seasonal cycle. Compared with the other regions, gelatinous zooplankton (jellyfish, salps, larvaceans, and ctenophores) were of minor importance. As with the inner shelf region, the early to midsummer decrease in zooplankton biomass was coincident with strong upwelling and high phytoplankton productivity. Upward displacement of subsurface isotherms and isohalines reached a maximum in June and July (Fig. 5), when surface layer nutrients were increasing and chlorophyll biomass was at its annual maximum (Fig. 6).

The outer shelf had the least seasonal variability in zooplankton biomass and the latest seasonal peak. Total biomass (annual average 4.6 g/m^2) was relatively constant from April through September. The highest total (about 6.5 g/m^2) was in August–September and lowest (about 2 g/m^2) in February. Calanoid copepods and chaetognaths were consistently the dominant taxa in spring and early summer. Salps (included with urochordates in Fig. 7 and 8) and were very abundant in late summer and autumn of several years but rare or absent in others.

Calanoid and cyclopoid copepods

Seasonal cycles of copepod biomass are shown in Fig. 9. The sampling and enumeration methods used in this study give very good estimates of abundance for the major species. Even the relatively rarer species included in the "subarctic," "southern," "other calanoid," and "other cyclopoid" categories are well censused where and when they make up more than a negligible fraction of the total copepod biomass. The regional and seasonal differences shown in Fig. 9 can therefore be interpreted with high reliability.

Four species are major contributors to the total copepod biomass in all regions. *Calanus marshallae, Pseudocalanus* spp. (mostly *P. mimus*, Frost 1989), and *Metridia pacifica* are important throughout the year and usually make up 50–80% of the copepod biomass. *Acartia longiremis* is important in summer and autumn. At the deeper locations only, "subarctic" copepods (strongly dominated by *Neocalanus cristatus* and *Neocalanus plumchrus*) make up 20–70% of the copepod biomass in spring and summer. Although they account for <10% of the total, "southern" copepods endemic to the Oregon and northern California coasts (e.g. *Clausocalanus parapergens, Mesocalanus tenuicornis*, and *Ctenocalanus vanus*) occur frequently at low to moderate numeric abundance from October through April.

The inner shelf and southern shelf regions share similar seasonal cycles, although biomass is consistently higher on the southern shelf. Peak biomass (strongly dominated by *C. marshallae* and *Pseudocalanus* spp.) is in late April to May and occurs slightly earlier on the inner shelf. These species decline rapidly in summer. *Acartia longiremis* biomass is maximum in July–August. *Metridia pacifica* (important only at deeper locations on the shelf) maintains a relatively constant biomass from April through September. All taxa except for those with southern affinities decline in autumn and winter; the southern taxa reach their maximum absolute and relative importance in February.

In the shelf break and slope region, the seasonal pattern is quite different. The peak of total biomass is of longer duration and is centered in summer rather than in spring. The spring and early summer copepod community is strongly dominated by the subarctic open-ocean species *N. cristatus* and *N. plumchrus*. These decline gradually through the summer, but the decline is largely offset by a July maximum of *C. marshallae* (the dominant species in the shelf regions in May–June). The "southern" group of calanoids occurs at moderate levels from October through May–June; midwinter levels are comparable with those found on the continental shelf, but the offshore population persists much longer into the spring and summer.

The depth distribution of the copepod population will be important in my subsequent discussion of the effects of horizontal advection. Total copepod biomass is concentrated both day and night in the upper 50 m at least from early spring through autumn (D. L. Mackas, unpublished). Of the species forming most of the local copepod biomass, only *M. pacifica* undergoes strong diel vertical migration. Several species within the "subarctic" group (*N. cristatus, N. plumchrus*, and *Eucalanus bungii*) seasonally migrate downward in summer or early autumn to depths >250 m but spend their spring and early summer development near the surface.

Chaetognaths

Seasonal cycles for the chaetognaths are shown in Fig. 10. Compared with the other major taxonomic groups, reliability

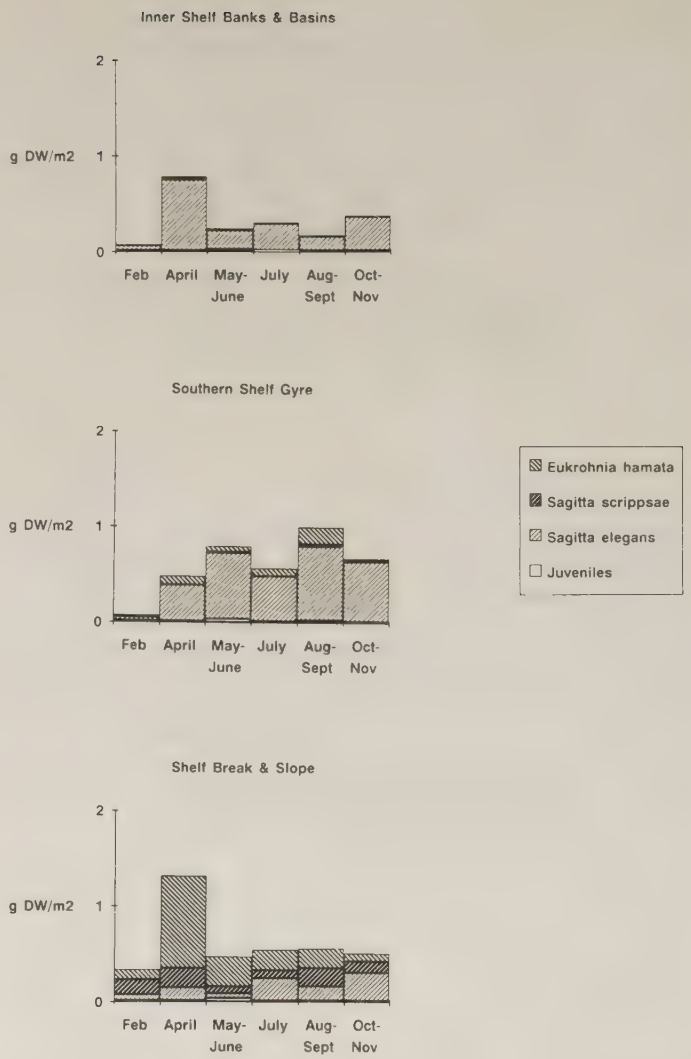

FIG. 10. Average seasonal cycles of chaetognath biomass off the outer coast of Vancouver Island. Spatial averaging is as in Fig. 7. The two shelf regions are strongly dominated by *Sagitta elegans* in all seasons. Offshore, *Eukrohnia hamata* and *Sagitta scrippsae* are dominant in winter and spring. The oceanic species are partially replaced by the inshore dominant *S. elegans* in summer and autumn, again suggesting seaward transport of zooplankton originating on the continental shelf.

of the regional and seasonal patterns is intermediate: poorer than for the copepods because of lower abundance per sample but better than for euphausiids and large gelatinous zooplankton because of much weaker within-region spatial and interannual variability. Nearly all of the chaetognath biomass is in one of four categories: undifferentiated juveniles (usually <5 mm in body length), *Sagitta elegans, Sagitta scrippsae*, and *Eukrohnia hamata*. An additional species endemic to more southerly parts of the California Current system, *Sagitta decipiens*, was recorded in some samples but was rare and probably not reliably differentiated from *S. elegans*. Total chaetognath biomass was low on the inner shelf and higher in the deeper water offshore (winter–spring) and on the southern shelf (summer–autumn). The major species all spend at least part of the day at depths >50 m.

The major between-region difference was in species composition. The chaetognaths caught on the continental shelf were almost entirely *S. elegans*. Seaward of the shelf break, *E. hamata* and *S. scrippsae* were much more abundant. This

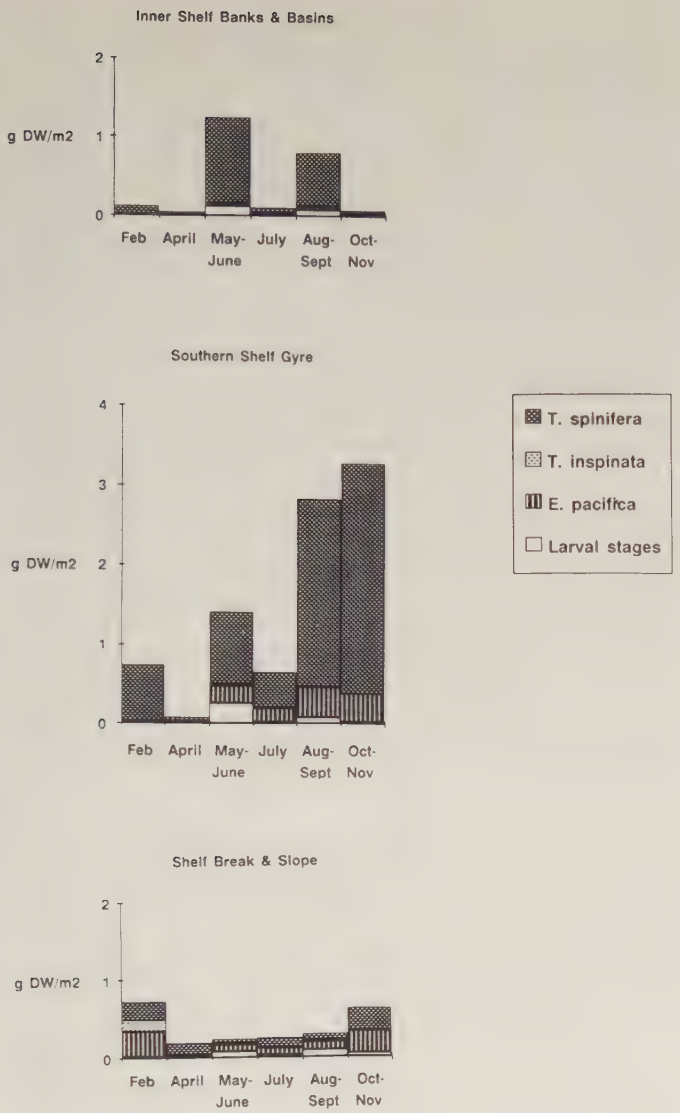

FIG. 11. Average seasonal cycles of euphausiid biomass off the outer coast of Vancouver Island. Spatial averaging is as in fig. 7. *Thysanoessa spinifera* is the dominant species on the continental shelf. *Euphausia pacifica* is dominant along and seaward of the shelf break and within the Juan de Fuca submarine canyon system. Larval stages show consistent abundance maxima in May–June and August–September. Seasonal time series for the juvenile and adult stages are unstable due to intense spatial patchiness; however, the euphausiids do not appear to share the strong spring maximum and summer decline of the shelf copepod community.

agrees with Lea (1955), who concluded from summer samples that *S. elegans* is indicative of coastal waters, while *S. lyra* (= *scrippsae*), *S. decipiens*, and *E. hamata* indicate offshore oceanic water. A new and interesting result in the present study is the seasonal shift in species composition in the offshore region. *Eukrohnia hamata* and *S. scrippsae* made up nearly all of the total from winter through spring, but were augmented by large numbers of *S. elegans* in summer and autumn.

Euphausiids

A dozen or more euphausiid species occur off the outer B.C. coast (Fulton and LeBrasseur 1984), but two abundant species (*Euphausia pacifica* and *Thysanoessa spinifera*) plus one less abundant species (*Thysanoessa inspinata*) account for nearly all of the euphausiid biomass in the La Perouse study area. Figure 11 shows the seasonal cycles for these three species plus undifferentiated larval stages. Of all the major taxonomic groups considered in this paper, the reliability of the regional and seasonal averages is lowest for the euphausiids because of their spatial patchiness, possible sampler avoidance, and year-to-year variability. The patterns shown in Fig. 11 must therefore be interpreted with caution. In particular, the strong late summer to autumn peak of euphausiid biomass on the southern shelf was observed only in two years and may not be a stable feature of the seasonal cycle.

Several results that were consistent across years can be identified. Euphausiid distributions off B.C. are very strongly related to bottom topography. Simard and Mackas (1989) mapped summer season euphausiid distributions using a high-frequency echo sounder. They consistently found the highest concentrations of all taxa in the immediate vicinity of steep depth gradients such as the shelf break and along the margins of banks and submarine canyons. Between-region differences in species composition are also tied to bathymetry. *Thysanoessa spinifera* was the only euphausiid common where bottom depths were shallower than about 150 m; conversely, *E. pacifica* (and occasionally *T. inspinata*) were the dominants at the deeper locations along and seaward of the shelf break and in the Juan de Fuca submarine canyon system. Adults and late juveniles of all three of the locally dominant euphausiid species feed near the surface at night and migrate downward in daylight. Daytime scattering layer depths vary with location from 100–150 m on the shelf to 150–200 m along and seaward of the shelf break but are frequently within the California Undercurrent upwelling "source water" (Simard and Mackas 1989).

All three regions showed two peaks in the abundance of larval euphausiids: May–June and August–September. Although the total larval biomass was small, these maxima are statistically stable and reliable because of the high numerical abundance and relatively weak within-region spatial variability of the euphausiid larvae. Because larvae were not identified to species, it is unclear whether the two peaks result from separately timed single spawning events by *E. pacifica* and *T. spinifera* or from repeated spawning by one or both of the major species.

Gelatinous zooplankton

Seasonal cycles of the dominant gelatinous zooplankton are shown in Fig. 12. Within this group, larvaceans, salps, and doliolids are filter feeders and are primarily herbivorous. Siphonophores, hydromedusae, and ctenophores are carnivorous and feed mostly on small crustaceans. Variability within the averaging units shown in Fig. 12 was again very large. Small-scale spatial patchiness was strong for all taxa. In addition, the salps and siphonophores showed extreme year-to-year differences. When present, they often made up a large fraction of the total zooplankton biomass.

The regions differed in composition and timing of peak biomass. On the inner shelf, the gelatinous zooplankton were mostly hydromedusae and ctenophores. Biomass peaked May–July and was relatively low August–April. The southern shelf region had low average levels throughout the year, although we occasionally observed high early summer concentrations of ctenophores. The offshore region had the highest concentrations of predatory gelatinous zooplankton in midsummer and the highest concentrations of salps in late summer and autumn.

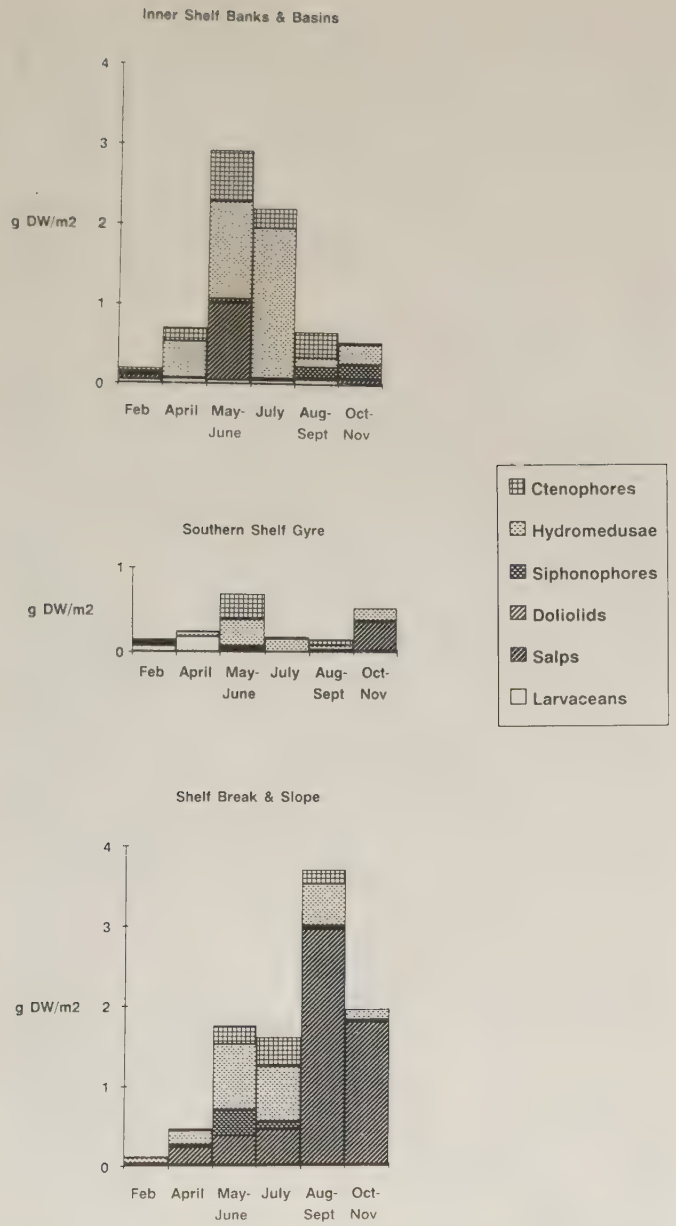

FIG. 12. Average seasonal cycles of gelatinous zooplankton off the outer coast of Vancouver Island. Spatial averaging is as in Fig. 7. Shelf regions are dominated by medusae and ctenophores. In some years only, the shelf break and slope region has extremely high biomass of salps, peaking in late summer and early autumn.

Zooplankton Population Balance

What sets the level of secondary production and zooplankton biomass off southwestern Vancouver Island? The potentially important biological and physical processes and rates include: (1) reproductive growth in population abundance, (2) somatic growth in individual body size, (3) mortality due to predation, and (4) active or passive immigration and emigration (swimming and transport by water motions).

To compare the relative importance of these, it is useful to consider the regional seasonal cycles in terms of a simple budget equation for the local rate of change of zooplankton biomass:

$$(1)\quad \partial Z_i/\partial t = (r + g - m)Z_i - \boldsymbol{u}\nabla(Z_i) + K\nabla^2(Z_i) + S$$

where Z_i is zooplankton biomass in taxonomic unit i; r, g, and m are taxon-specific per capita rates of reproduction, somatic growth, and mortality; $\boldsymbol{u}$ is the advective velocity vector; $\nabla(Z_i)$ is the spatial gradient of Z_i; K is an eddy diffusivity coefficient, and S is net active immigration or emigration. The reproductive and somatic growth terms are always net gains and should covary positively with food availability. The predation mortality term is always a net loss; its absolute magnitude should covary with predator abundance and biomass. The passive (advective and diffusive) and active (swimming) immigration terms can be either positive or negative. The advection term is a gain if upstream concentrations are high compared with local concentrations and a loss if upstream concentrations are low. Similarly, the diffusive term is a gain if zooplankton concentrations in neighboring regions are higher than the local concentration and a loss if they are lower. Compared with the potential magnitude of the other terms, active horizontal migration can be assumed negligible for zooplankton (Although active vertical movement may interact with vertically sheared flow fields to affect net horizontal advective input or loss).

In the remainder of this paper, I will examine how the various terms in equation (1) vary between regions and seasons. The goal here is not to solve equation (1). Rather, I will be comparing the seasonal pattern of observed $\partial Z/\partial t$ for various taxa with literature-derived estimates of the time- and region-dependent sign and magnitude of growth, mortality, and mixing rates. This will allow me to estimate by difference the sign and magnitude of the advection term.

Net rate of biomass change

I first consider the net local rate of population change $\partial Z/\partial t$. The major observation from the regional time series is that the zooplankton populations increase in spring and early summer and begin to decline in summer or early autumn. The season of net increase of zooplankton biomass ends earliest nearshore and persists longest offshore. For the inner shelf region, herbivorous components of the zooplankton increase rapidly (an exponential rate of about 1.3%/d) from February through June and then decrease July–November (1.5–2%/d). Carnivorous taxa differ only slightly. Chaetognaths begin to decline earlier (April to May–June) and hydromedusae later (July to August–September). The overall rate of decline of zooplankton biomass is at least as rapid in midsummer as it is in late autumn or winter.

In the southern shelf gyre region, taxa that live mostly in the upper 10–50 m (calanoid copepods, hydromedusae, ctenophores, and pteropods) increase from winter through late spring and then (as in the inner shelf region) decline through summer and autumn. Taxa that migrate to greater depths (euphausiids and chaetognaths) do not show a pronounced summer decline but instead either persist or increase from June through November.

In the shelf break and slope region, total zooplankton biomass increases rapidly from February through April and then (unlike the more inshore locations) continues to increase slowly through August–September. Calanoid copepod biomass begins to decline at about the end of July. This is significantly later than the copepod decrease in the inner shelf regions despite the relatively greater importance in the offshore copepod community of deep seasonal migrators such as *Neocalanus* and *Eucalanus* that leave the upper 250 m in mid- to late summer. The late summer decline of herbivorous copepods is offset in some years (1983, 1985, and 1987) by a large increase in salp biomass.

Food availability and potential growth

When and in what subregions was food availability adequate to support sustained high zooplankton growth rates? For herbivorous zooplankton (most of the total biomass within the copepod, euphausiid, urochordate, and pteropod taxonomic categories) the answer appears to be most of the spring and summer seasons in all regions. Phytoplankton biomass was generally high but was particularly rich from mid- to late summer on the continental shelf. Food quality, as influenced by biochemical composition of the phytoplankton (Harrison et al. 1990; Thompson et al. 1990), was not estimated directly in this study but was probably high given the ample availability of light and dissolved nutrients.

How does the availability of phytoplankton compare with the metabolic and growth needs of the zooplankton? Huntley and Boyd (1984) estimated maintenance and growth-saturating food concentrations (micrograms carbon per litre) as a function of water temperature and zooplankton body size. For the 9–13°C seasonal temperature range in the euphotic zone off B.C. (Fig. 5), Huntley and Boyd's "maintenance" food levels are about 40–50 μg C/L and their "saturating" food levels about 175–200 μg C/L. To compare phytoplankton biomass off B.C. with these requirements, I have applied an assumed C:chlorophyll ratio of 40:1 to the chlorophyll time series in Fig. 6. The lowest chlorophyll concentrations were observed from October to February (0.5–1 mg Chl/m^3 = about 20–40 μg C/L). This annual minimum level therefore provides a near-maintenance ration in all regions; it would be supplemented by availability of nonpigmented food (microzooplankton and detritus). Seaward of the shelf break the phytoplankton concentration from spring through autumn is usually above the maintenance level but below growth-saturating levels (typically 1–2 mg Chl/m^3 = 40–80 μg C/L). Phytoplankton concentrations below those required for herbivore maintenance occur only in late spring immediately after the spring bloom. Food availability is usually much higher on the continental shelf and along the shelf break (Fig. 6). Average phytoplankton concentrations from March through September are often at or above the estimated saturation level (typically 3–8 mg Chl/m^3 = 120–320 μg C/L).

Huntley and Boyd (1984) tabulated published measurements of the temperature-dependent food-saturated growth rate of small zooplankton and fitted the equation

$$(2) \quad G'_{max} = 5.42\, e^{0.110T}$$

where G'_{max} is maximum mass-specific food-saturated growth rate (percent per day) and T is temperature (degrees Celsius). This equation ignores species and body size effects but predicts growth rates close to those measured for the locally dominant copepod genera *Calanus* and *Pseudocalanus* (Vidal 1980; Peterson 1986) and is therefore probably a good approximation to a biomass-weighted average for all herbivorous copepods. For the 9–13°C euphotic zone temperatures observed off B.C. (Fig. 5), predicted G'_{max} ranges from 14.5 to 22.5%/d. Because phytoplankton availability off the B.C. coast is generally high from spring through autumn (Fig. 6), per capita zooplankton growth rates (biomass specific $r + g$) should often be a large fraction of this predicted physiological maximum. Weight-specific growth at phytoplankton concentrations between the maintenance and saturating levels can be estimated from a modified von Bertalanffy equation (Huntley and Boyd 1984):

$$(3) \quad r + g = 1/W\,(\partial W/\partial t) = a\,C\,b\,W^{n-1} - k\,W^{m-1}$$

where W is zooplankton body dry weight (milligrams), C is phytoplankton biomass estimated from average chlorophyll a concentration (milligrams per cubic metre), a is assimilation efficiency (assumed = 0.7), and b, n, k, and m are temperature-dependent coefficients fitted by Huntley and Boyd (1984) but converted to the biomass and rate units used in this paper. Growth rates of herbivorous zooplankton are near zero during the winter and perhaps in the offshore region in May–June (immediately after the spring bloom and prior to the upwelling season). During the spring bloom, predicted growth rates are about 6–9%/d in all regions. On the continental shelf, they should increase relatively steadily to a summer maximum >15%/d. In the offshore region, there is a minimum in food availability following the spring bloom (predicted growth rates 0–5%/d) followed by an increase to a late summer maximum (predicted growth about 8–10%/d near the shelf break decreasing seaward to about 3–6%/d).

From spring through autumn, this regional and seasonal pattern of food availability and potential growth of herbivorous zooplankton provides a very poor match to the regional time series of $\partial Z/\partial t$ described in the previous section. Based on food availability, the ranking of predicted population growth potential is as follows (from highest to lowest): continental shelf regions, May–September; all regions, March–April; offshore region, July–September; offshore region, October–February; offshore region, May–June; continental shelf regions, October–February. Net zooplankton population change was strongly negative in the region and season of highest food availability (inner shelf, July–September) and strongly positive in a region and season of moderate to low food availability (offshore, late spring and early summer). In the summer season, processes other than food-limited per capita growth rate must therefore be important.

Predation mortality

Predation on the zooplankton community can be divided into two components: vertebrate predation (mostly by finfish) and invertebrate predation (mostly by chaetognaths, medusae, and ctenophores). Euphausiids and salps may also prey on small zooplankton, but their role is difficult to estimate. The dominant euphausiid species in the region (*E. pacifica* and *T. spinifera*) are believed to be primarily herbivorous in local waters (Simard and Mackas 1989).

Most of the regional finfish biomass is pelagic and is made up of four genera (D. Ware and G. A. McFarlane, PBS, pers. comm.): Pacific hake (*Merluccius productus*), Pacific herring (*Clupea harengus*), spiny dogfish (*Squalus acanthias*), and the various Pacific salmon (*Oncorhynchus* spp.). Of these, only the dogfish are year-round residents. Dietary analyses (Tanasichuk et al. 1991) show that these fish prey heavily on euphausiids but relatively lightly on the other zooplankton. Annually integrated predation on euphausiids appears to significantly exceed the instantaneous euphausiid biomass (D. Ware, PBS, pers. comm.) and is highly seasonal (high from early summer through autumn, low in the remainder of the year) because of strong alongshore and cross-shore migration into and out of the region by the pelagic fish stocks. From May through October, the mortality rate imposed by finfish on euphausiids is almost certainly in the range 1–10%/d (I will assume about 3%/d); based on this, the mortality rate imposed on all other zooplankton is unlikely to exceed 1%/d.

In contrast, invertebrate predation is heaviest on small crustaceans such as copepods and euphausiid larvae. Weight-spe-

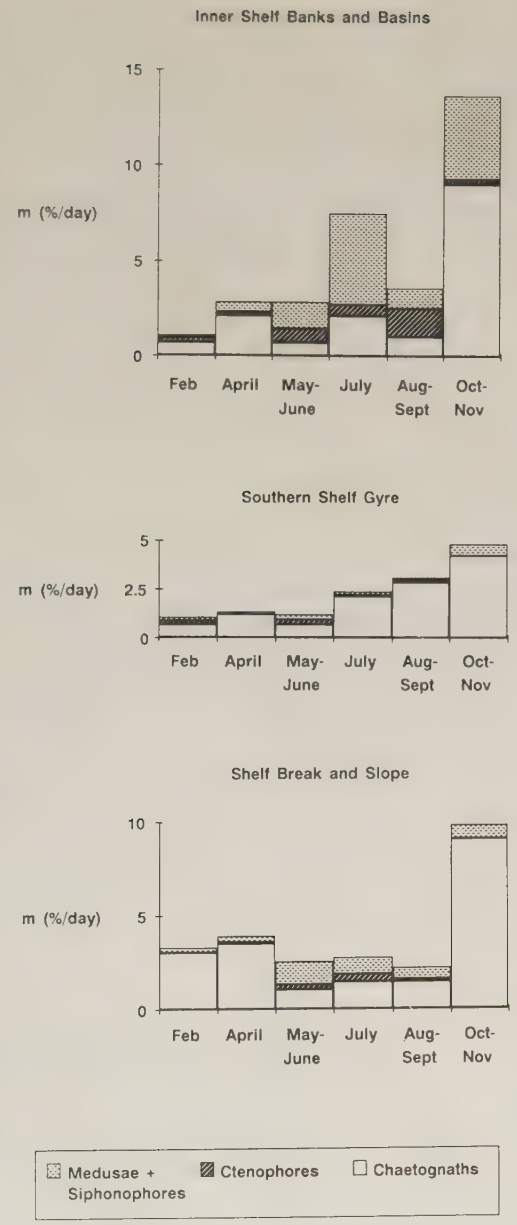

FIG. 13. Estimated rate of mortality (m, percent per day) of small zooplankton imposed by invertebrate predators, based on published estimates of predator ration. Spring and summer season mortality rates are mostly in the range 1–5%/d. The high rates in October–November may be an artifact of applying an assumed constant predator ration in a season of unusually low prey abundance.

cific ration estimates (prey eaten per unit predator biomass per day; data from Reeve (1980), Pearre (1981), and Larson (1987) converted to a dry weight basis are about 3%/d for medusae and ctenophores and about 7%/d for chaetognaths. Multiplication by the water column ratio of predator to prey estimated dry weight biomass (from Fig. 7) provides the estimates of predation mortality shown in Fig. 13. The estimates shown in Fig. 13 assume that achieved predator ration is independent of prey density within the local seasonal range (0.5–5 g dry weight/m^2); if this assumption is incorrect, winter and inner shelf mortality rates are likely to be lower than shown and spring and offshore summer season rates perhaps slightly higher. Regional and seasonal differences in pathway (chaetognaths versus ctenophores versus medusae) are apparent in Fig. 13: chaetognaths are the dominant predator in winter and offshore, while medusae are more important in summer and on the inner shelf. The estimated total loss rate to predation varies from about 1%/d (southern shelf region in spring and early summer) to about 10–15%/d (inner shelf and offshore regions in autumn). From spring through autumn, the overall average is 2–3%/d. This is substantial but appears to be insufficient to offset the probable growth rates of the small zooplankton in all except the October–November time period (when predation mortality may be overestimated).

Horizontal mixing

Net effects of horizontal mixing can be quantified only very roughly. Between-region differences in zooplankton concentration are about 25% of grand average biomass. Spatial separation between region centers is about 50 km. The normalized spatial gradient ($\nabla(Z_i)\cdot Z_i^{-1}$) is therefore approximately 0.5×10^{-5}/m, and the square of the gradient is approximately $0.25 \times 10^{-10}/m^2$. An assumed horizontal diffusivity coefficient K of 5×10^3 m^2/s gives a predicted biomass specific exchange rate of about 1%/d. Although the uncertainty of this estimate is large, the magnitude is almost certainly smaller than the rates of growth and invertebrate predation mortality. Furthermore, despite uncertainty in magnitude, the local sign of the net exchange due to mixing can be clearly identified: the tendency should be to equalize biomass and composition ratios between regions. The inner shelf region should therefore gain biomass from both regions from July through April (net loss possible only in May–June). The offshore region should gain in May–June but lose total biomass from February to April and from July to September. The southern shelf region should lose biomass overall; the major transfer should be to the offshore region in May–June and to the inner shelf for the remainder of the year.

As before, the match to observed regional and seasonal $\partial Z/\partial t$ is poor. The inner shelf zooplankton biomass decreases rapidly in summer despite expected mixing inputs from the richer adjoining regions. The offshore region biomass continues to increase in summer despite probable mixing losses to the continental shelf banks.

Horizontal and vertical advection

Both direct evidence and process of elimination indicate that net advection plays an important role in zooplankton population dynamics off the B.C. coast. The various water property time series all show large summer season inputs of upwelled water and "new" nitrogen: increasing salinity in all regions from April to September coinciding with decreasing surface temperatures in the shelf regions from July to September and high average levels and June–September increases in nitrate and chlorophyll concentration. The upwelling signal is strongest and earliest on the continental shelf but also appears as July–September increases in salinity, nitrate, and chlorophyll seaward of the shelf break. For as long as the upwelling continues (roughly April–September), continuity requires ongoing alongshore and offshore displacement of the previously resident water (and its associated chemical and biological tracers). However, it is important to remember that alongshore components are much larger than cross-shore components, and recently upwelled water from the California Undercurrent forms only a 10–20% fraction of the total upper layer flow (see source mixture isopleths in Mackas et al. 1987). The "upstream" source for the VICC on the inner shelf is the upper layer discharge from Juan de Fuca Strait. This in turn is a tidally stirred mixture of upwelled California Undercurrent water and Strait of Geor-

gia surface water. "Upstream" for the southern Vancouver Island offshore region is the Shelf Break Current which forms as a mixture of upwelled and VICC water on the (narrow) outer continental shelf at the north end of the island (Denman et al. 1989).

What effects of advection can be seen within the zooplankton community? Various shifts in community composition suggest net offshore transport of neritic and shelf species: note the progressive summer season replacement in the shelf break and slope region of "offshore" chaetognaths *E. hamata* and *S. scrippsae* by the "shelf" species *S. elegans* and of "offshore" copepods with both northern (*Neocalanus* spp.) and southern affinities (e.g. *Mesocalanus, Clausocalanus*, and *Ctenocalanus*) by the "shelf" dominants *C. marshallae* and *Pseudocalanus* spp. During the April–September upwelling season, there does not appear to be a comparable shoreward transport or exchange of "offshore" species. *Eukrohnia hamata* and *S. scrippsae* remain rare on the shelf throughout the year. Gelatinous zooplankton that are important throughout the summer seaward of the shelf break (salps and siphonophores) occur more frequently on the continental shelf after the fall transition to downwelling circulation. Copepod species with southern affinities are relatively common offshore in every month except July and August; they are found on the shelf *only* after the fall transition and before the spring transition.

Probable per capita rates of growth, predation mortality, and mixing exchange do not adequately explain the observed seasonal changes in zooplankton biomass (in particular the sharp summer decrease in the food-rich continental shelf region coupled with a sustained population increase in the offshore region). However, these seasonal trends are consistent with a hypothesis of high loss from the shelf due to dilution (and advective displacement to the offshore region) of the spring and early summer resident populations by water containing relatively little zooplankton. Direct quantification of this potential loss (input) rate is difficult. Watermass analysis using chemical tracers (Mackas et al. 1987) gives good identification of subsurface source water types. We know from this analysis that much of the shelf water below 50 m is displaced seasonally by nearly pure California Undercurrent water and that a substantial amount of upwelled water reaches the surface layer either through local upwelling and mixing or via the Juan de Fuca estuarine discharge. We also know that this water originates at considerable depth (200–300 m, Freeland and Denman 1982) south of the study area and is very unlikely to contain high biomass of any taxa other than (perhaps) euphausiids and chaetognaths. Differences between taxa are again suggestive. June–September changes in biomass within the shelf zooplankton community are strongly negative for surface layer inhabitants such as most of the calanoid copepods and much less negative or weakly positive for taxa with greater depth range such as *Metridia, Thysanoessa*, and *Sagitta*. We do not know the magnitude of the vertical component of the advective velocity $\boldsymbol{u}$. The upward displacement rate of water property isoclines (about 0.2–0.5 m/d) gives only a minimum estimate of net vertical velocity because some of the previously upwelled water is also being displaced laterally. We also do not have adequate knowledge of large-scale horizontal gradients and their interaction with alongshore advection by the Shelf Break Current and the VICC.

Lacking direct measurement of the advective gain (or loss) term, it is still possible to ask how big it needs to be to balance equation (1), given conservative estimates (minimal for gains, maximal for losses) for the magnitude of the other terms in the equation. In Table 2, I make this rate comparison for copepod biomass in each of the three La Perouse zooplankton averaging regions for February–May (the period of net increase in all regions) and for June–July (rapid net decrease on the shelf, near zero net change offshore). Units are estimated per capita rate of change (percent per day). Net rate of change ($\partial Z/\partial t$) is derived from the copepod biomass time series shown in Fig. 9 by assuming for each region a constant exponential rate of increase or decrease between the start and end of each time interval. Per capita copepod growth rate ($r + g$) is estimated from equation (3) using time- and region-averaged euphotic zone temperature and phytoplankton concentration. However, to be conservative, the growth rate estimates in Table 2 are intentionally biased low by about 25% from those predicted using the coefficients fitted by Huntley and Boyd (1984). Average mortality due to invertebrate predation (from Fig. 13) is estimated to be in the range 2.5–6%/d; I assume that vertebrate predation accounts for an additional 1%/d in all regions in summer when the biomass of migratory fish is high. Horizontal mixing between regions is assumed to add about 1%/d to the low biomass of the inner shelf and remove about 0–1%/d from the higher offshore and southern shelf zooplankton biomass.

Given the above estimates, there must be a large advective loss of surface layer zooplankton from the inner shelf region during the spring through autumn productive season (about 2.5–7.5%/d in spring increasing to about 5–11%/d in summer). The

TABLE 2. Estimated magnitude of various components of the population turnover rate for copepod biomass in selected regions off the B.C. outer coast in spring and summer. Error bars are approximate estimates of the computational uncertainty for each term. Net change $\partial Z/\partial t$ is estimated from seasonal time series, growth rate $r + g$ from environmental temperature and food availability, mortality rate m from predator biomass, mixing rate $K\nabla^2 Z$ from between-region spatial gradients, and advection rate $\boldsymbol{u}\nabla Z$ by difference.

		Estimated turnover rates (%/d)				
Season	Region	$\partial Z/\partial t$	$r + g$	m	$K\nabla^2 Z$	$\boldsymbol{u}\nabla Z$
Spring (March–May)	Inner shelf	1.3 ± 0.2	7 ± 2	−2 ± 1	1 ± 0.5	−4.7 ± 2
	Banks and basins					
	Southern shelf gyre	2.3 ± 0.2	7 ± 2	−1.3 ± 1	−1 ± 0.5	−2.5 ± 2
	Shelf break and slope	1.4 ± 0.2	4 ± 3	−4 ± 1	0 ± 0.5	1.4 ± 3
Summer (June–July)	Inner shelf	−2.0 ± 0.2	12 ± 3	−7 ± 2	1 ± 0.5	−8 ± 3
	banks and basins					
	Southern shelf gyre	−2.0 ± 0.2	12 ± 3	−3 ± 1	−1 ± 0.5	−10 ± 3
	Shelf break and slope	0.0 ± 0.2	6 ± 3	−3.5 ± 1	−1 ± 0.5	−1.5 ± 3

southern shelf gyre region data indicate low to moderate advective loss (about 0–5%/d) in spring but a very high washout rate (7–13%/d) in summer. The offshore region data indicate a small net advective input in spring (when potential inshore source regions have relatively high zooplankton biomass) and a small net advective loss in summer (when upwelling circulation is strongest but copepod biomass in inshore source waters is greatly reduced). In all regions, the advective component of population turnover appears to be at least comparable with the component caused by local predation mortality; in the shelf regions, summer advective losses appear to be much larger than losses to predation.

The net effect of the spring through autumn upwelling circulation on zooplankton is of course very dependent on the vertical distribution of each zooplankton species and of current direction (Peterson et al. 1979). The late spring through summer decline of total zooplankton biomass on the Vancouver Island continental shelf is mostly due to the decrease in the populations of surface layer inhabitants (mostly copepods). Although the seasonal time series for deeper dwelling and migratory taxa such as chaetognaths and euphausiids are less reliable because of patchiness and sample size problems, these taxa tend to maintain or increase their spring population levels at the shelf locations deep enough to include their normal daytime depth range (Fig. 10 and 11). It is therefore plausible that the net effect of the summer upwelling circulation on euphausiids and chaetognaths is to accumulate rather than disperse them from the continental shelf. This explanation seems particularly likely given the high summer season predation mortality on euphausiids imposed by migratory finfish.

Summary

(1) Zooplankton biomass and species composition in continental shelf and slope waters off the southwest coast of British Columbia show a strong seasonal cycle (three- to sixfold variation for total biomass, much larger for individual taxa).

(2) A relatively small number of taxa account for most of the zooplankton biomass: the copepods *Calanus marshallae*, *Pseudocalanus* spp., *Neocalanus cristatus* and *plumchrus*, and *Metridia pacifica*; the euphausiids *Euphausia pacifica* and *Thysanoessa spinifera*; the chaetognaths *Sagitta elegans* and *Eukrohnia hamata*; *Salpa* spp.; the hydromedusae *Aglantha* and *Phialidium*; and the ctenophore *Pleurobrachia*.

(3) Within the overall study area, at least three subregions can be clearly identified based on bathymetry and current pattern. Although the occurrence of zooplankton species is very similar in all three regions, total amount, percent composition, and seasonality differ sharply, as do temperature, salinity, and nutrient and phytoplankton concentrations.

(4) From spring through autumn, there are large inputs of upwelled water (and nutrients) to the continental shelf. Phytoplankton biomass is high, especially in mid- to late summer, and there is ample food to support the growth of herbivorous zooplankton.

(5) Despite high food availability and relatively low predation pressure, surface layer zooplankton populations decline rapidly on the continental shelf from late spring through autumn. Offshore zooplankton populations increase slowly during the same time period. A high washout rate due to upwelling and subsequent seaward and alongshore transport is the most plausible explanation. The advective component of population turnover rate appears to be larger than local predation mortality.

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Use of Risk Analysis to Assess Fishery Management Strategies: A Case Study using Orange Roughy (*Hoplostethus atlanticus*) on the Chatham Rise, New Zealand

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Francis, R. I. C. C. 1992. Use of risk analysis to assess fishery management strategies: a case study using orange roughy (*Hoplostethus atlanticus*) on the Chatham Rise, New Zealand. Can. J. Fish. Aquat. Sci. 49: 922–930.

Risk analysis can enhance the value of scientific advice to fishery managers by expressing the uncertainty inherent in stock assessments in terms of biological risk. I present a case study involving an overexploited population of orange roughy (*Hoplostethus atlanticus*) on the Chatham Rise, New Zealand. This analysis quantifies the risk to the fishery and shows how this decreases as the rate of reduction in total allowble catch increases. The technique helps fishery managers balance biological risk against economic risk. Ways of generalizing the technique are discussed.

Une analyse des risques peut améliorer la valeur des conseils scientifiques fournis à des gestionnaires des pêches parce qu'elle exprime l'incertitude propre aux évaluations de stock en termes de risques biologiques. L'auteur présente une étude de cas portant sur une population surexploitée d'hoplostète orange (*Hoplostethus atlanticus*) peuplant le massif Chatham, en Nouvelle-Zélande. L'analyse quantifie le risque pour la pêche et montre comment il diminue en fonction d'une augmentation du taux de réduction du total des prises allouées. Cette méthode aidera les gestionnaires des pêches à équilibrer le risque biologique et le risque économique. L'auteur examine aussi divers moyens de généraliser cette méthode.

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Fishery managers have the difficult task of evaluating scientific information and assessing its implications for fishery management decisions. What makes this task particularly difficult is the uncertainty associated with stock assessments. For example, suppose the Total Allowable Catch (TAC) for a particular population is 6000 t. What should the manager do if the estimated optimal yield level is 4000 t, with a 95% confidence interval of 2700–6900 t? Is this sufficient evidence to warrant a reduction in TAC? Even if the 95% confidence interval were narrow enough to lie completely below the current TAC (say, 3200–5400 t), how is the manager to judge how urgent the need is to reduce the TAC? Would the fishery be liable to suffer long-term harm if, in order to allow the fishing industry time to adjust fishing patterns and marketing obligations, the reduction in TAC were delayed for several years?

The managers' task may be made easier if uncertainty in a fishery assessment were expressed in terms of risk to the fishery under different management options (Peterson and Smith 1982; Linder et al. 1987). This paper describes a risk analysis for the orange roughy (*Hoplostethus atlanticus*) fishery on the Chatham Rise, New Zealand, and discusses ways of generalizing the technique.

Background to the Fishery

Orange roughy are trawled below about 750 m on the Chatham Rise, to the east of New Zealand, mostly between mid-June and mid-August — immediately before, during, and after the spawning aggregations appear. The fishery started in the early 1980s, and since the mid-1980s the reported catch has averaged about 30 000 t; the TAC for 1989–90 was 28 637 t (the quota year runs from October to September).

In recent years, it has become clear that this rate of harvesting is not sustainable. Mace et al. (1990) showed that the productivity of orange roughy was much lower than had previously been thought, and an annual series of trawl surveys during the spawning season showed a rapid decline in biomass (see below). Further evidence for such a decline was presented by Smith et al. (1991). They showed that between 1982 and 1988, there was a substantial loss of genetic diversity in orange roughy on the Chatham Rise and suggested that this was caused by a large decrease in biomass due to fishing, and differential rates of mortality for homozygous and heterozygous fish.

At the time of the assessment described here (between the 1989 and 1990 spawning seasons), it was widely agreed that a reduction in TAC was required. The questions to be answered were "to what level should the TAC be reduced?" and "at what rate?" Prior to the assessment, there was an expectation that TAC would be reduced at 5000 $t \cdot yr^{-1}$.

Biomass and Yield Estimation

Estimation of Virgin Biomass

The biomass estimation procedure is based on a deterministic age-structured population model (Appendix I) with parameters as in Table 1. The values used for natural mortality and stock–recruit steepness are educated guesses. To allow a reasonable percentage of 1-yr-old fish to reach maturity, natural mortality

TABLE 1. Orange roughy life history parameters used in the population model. Growth and weight–length parameters are from Mace et al. (1990). Age at entry to the fishery (=age at maturity because the fishery is targetted predominantly on spawning fish) was derived from an estimated length at entry to the fishery of 32 cm. Details of the model are described in Appendix I.

von Bertalanffy growth parameters:	L_∞ = 42.5 cm, k = 0.059·yr^{-1}, t_0 = −0.346 yr [Length (cm) = L_∞ (1−exp(−k(age (yr) − t_0)))]
Natural mortality:	M = 0.05·yr^{-1}
Weight–length parameters:	a = 0.0963, b = 2.68 [Weight (g) = a(length (cm))^b]
Age at maturity:	A_m = 23 yr
Age of entry to the fishery:	A_f = 23 yr
Stock–recruit steepness:	h = 0.95
Recruitment variability:	σ_R = 1.2

TABLE 2. Estimated catch history, trawl survey biomass indices, and CVs. Catches are for October–September years and include a 30% allowance for unreported mortality. Trawl surveys were carried out in July, a time by which approximately half the annual catch has been taken.

Year	Catch (t)	Trawl survey index	CV
1978–79	15 340		
1979–80	40 430		
1980–81	36 660		
1981–82	32 354		
1982–83	20 064		
1983–84	32 263	164 835	16
1984–85	38 142	149 425	15
1985–86	39 098	102 975	16
1986–87	39 896	80 397	15
1987–88	31 478	97 108	25
1988–89	42 621	66 291	18
1989–90	37 228[a]		

[a]Projected.

could not be much higher than 0.05; a steepness of 0.95 reflects the idea that recruitment strength is much more dependent on environmental factors than on the size of the parent stock (see Appendix I for a definition of steepness). The sensitivity of our calculations to these parameters is examined in a later section.

A complete catch history for the population and a time series of biomass indices from trawl surveys are presented in Table 2.

The maximum likelihood method was used to estimate B_0, the virgin biomass of the population, and q, the catchability constant relating the trawl survey biomass indices to absolute biomass. The statistical assumptions used were that each biomass index, O_i, is normally distributed with expected value equal to q times the true biomass, B_i, and that all biomass indices have the same coefficient of variation (CV), c.

The log-likelihood of observing the biomass indices, O_i, is given by

$$(1)\quad \lambda = -0.5n\log(2\pi) - n\log(c) - n\log(q) - \Sigma_i\log(B_i) - 0.5c^{-2}\,\Sigma_i(O_i/(qB_i)-1)^2$$

where n is the number of biomass indices.

The maximum likelihood estimates, $\hat{q}$ and $\hat{c}$, are

$$(2)\quad \hat{q} = (1/n)\Sigma_i(O_i/B_i)$$

$$(3)\quad \hat{c}^2 = (1/n)\Sigma_i(O_i/(\hat{q}B_i)-1)^2$$

and the maximum likelihood estimate, $\hat{B}_0$, is that which maximizes

$$(4)\quad \lambda = -n\log(\hat{c}) - n\log(\hat{q}) - \Sigma_i\log(B_i).$$

To obtain an unbiased estimate of c, the n in (3) was replaced by $(n-2)$. (The maximum likelihood estimate of c was used in estimating B_0.)

Since the B_i can be calculated from B_0 (using the catch history and age-structured model), $\hat{q}$,$\hat{c}$, and λ are all functions of B_0. Thus, (4) allows the estimation of B_0 by a one-dimensional search rather than the three-dimensional search that would be required if (1) were used directly. The maximum likelihood estimates were $\hat{B}_0$ = 411 000 t and $\hat{q}$ = 0.59.

The trawl surveys' CV, c, was estimated as 15%. This is probably an underestimate, as it is lower than all but two of the estimates in Table 2 and these, because of the two-phase methodology of Francis (1984), will tend to be underestimates. However, an upper bound for c may be calculated using the fact that the CV of a two-phase survey is known to be lower than that of a conventional survey with the same number of stations (Francis 1984). For each of the surveys in Table 2 the quantity $c_1(N/N_1)^{0.5}$ was calculated as an estimate of the CV that would have been obtained had conventional surveys been used (where c_1 is the CV, and N_1 is the number of stations, for phase 1 of the survey, and N is the total number of stations in the survey). The median of these estimates, 19%, was taken as an upper bound for c.

Estimation of a Probability Distribution for B_0

For the risk analysis, we need a probability distribution for B_0. The following Monte Carlo simulation procedure provides this. These simulations answer the question "If this were the true B_0, how likely is it that we would get an estimate of virgin biomass as large as or greater than $\hat{B}_0$?"

The procedure was as follows.

(1) Choose a trial value of B_0.

(2) Calculate the biomass history for this B_0.

(3) Generate simulated trawl survey indices (assuming that the indices are normally distributed with mean value equal to the corresponding value in the biomass history, and with CV c).

(4) Calculate the maximum likelihood estimate $\hat{B}_{0sim}$ for the simulated survey indices.

(5) Repeat steps 3 and 4 one hundred times and calculate the proportion, p, of the $\hat{B}_{0sim}$ that are greater than $\hat{B}_0$.

(6) Repeat steps 1–5 for a range of trial B_0 values.

(7) Graph p against the trial B_0 values and draw a smooth line through the points.

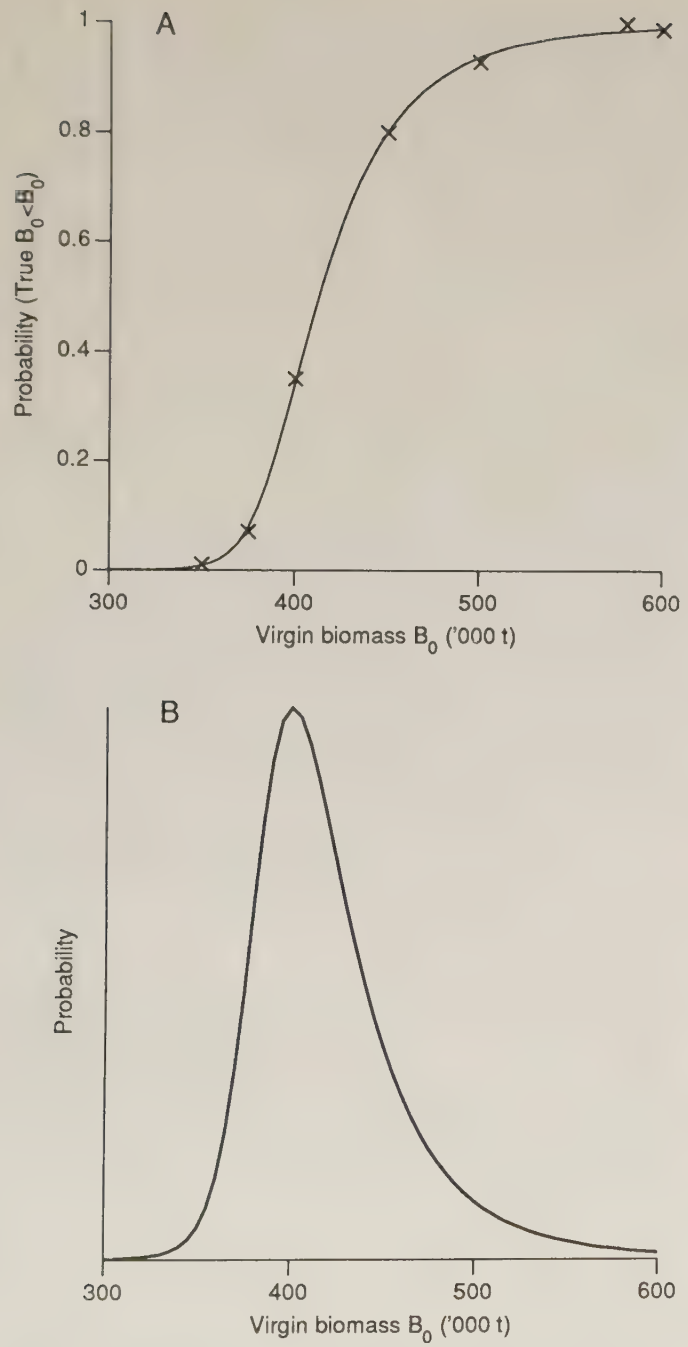

FIG. 1. Estimated probability distribution for B_0. (A) Estimates of the probability that the true B_0 is less than given trial values (×) and the cumulative probability distribution (fitted curve); (B) the probability density function for B_0.

The line drawn in step 7 is the estimated cumulative distribution function for B_0 (Fig. 1A). That is, the height of the curve above any trial value of B_0 is an estimate of the probability that the true value of B_0 is less than this value. The smooth curve used was the cumulative distribution function of a Johnson's S_U distribution (Johnson and Kotz 1970, p. 23) which was fitted by maximum likelihood (Appendix II).

The derivative of this curve is a probability distribution for B_0 (Fig. 1B). Cox called this type of distribution a confidence distribution (Kendall and Stuart 1967, p. 102). The area under the curve between two values of B_0 is proportional to our confidence that the true B_0 lies between these two values. The procedure for estimating p for each B_0 is analogous to Mood and Graybill's (1963, p. 256) "general method for obtaining confidence intervals."

TABLE 3. Percent changes in estimated yield and biomass for given values of population model parameters. Changes of <0.5% are given as +0 or −0, according to the direction of the change. Notation as in Table 1.

Change in parameters	Change in MCY (%)	Change in biomass (%)	
		Virgin biomass (B_0)	1990–91 biomass (B_{curr})[a]
L_∞ + 2.11, k − 0.006[b]	−4	+1	+0
L_∞ − 2.11, k + 0.006[b]	+4	−1	−0
A_f + 1, A_m + 1	+2	−0	−0
A_f − 1, A_m − 1	−2	+1	+0
M + 0.01	+17	−4	−2
M − 0.01	−17	+4	+1
h = 0.99	+9	—[c]	—[c]
h = 0.75	−25	—[c]	—[c]

[a]Beginning of season.
[b]These changes in L_∞ and k represent ±1 standard error.
[c]Nil.

TABLE 4. Orange roughy biomass and yield estimates based on a range of values of M (0.05 is considered the most likely value of M). For all values of M, the estimate of c, the CV of the trawl surveys, was 15%.

Natural mortality M	Virgin biomass B_0 (t)	Catchability q	1990–91 biomass B_{curr} (t)[a]	MCY (t)
0.025	452 000	0.52	92 000	4 200
0.05	411 000	0.59	80 000	7 500
0.075	372 000	0.67	77 000	10 500
0.1	336 000	0.77	74 000	13 100

[a]Beginning of season.

Yield Estimation

The New Zealand Ministry of Agriculture and Fisheries has adopted as a reference yield the concept of the Maximum Constant Yield (MCY), which is defined as the maximum constant catch that is estimated to be sustainable, with an acceptable level of risk, at all probable future levels of biomass. For the Chatham Rise orange roughy fishery, this was chosen as the target level towards which TACs should be reduced.

MCY was estimated using the rule of thumb that MCY = 2/3 MSY where MSY is the deterministic maximum sustainable yield.

Using a yield per recruit analysis coupled with the Beverton and Holt stock–recruit relationship (and the life history parameters in Table 1) the MSY for orange roughy was estimated as 2.7% of B_0, so the target level for TAC reduction was set at 7500 t (= (2/3) × 0.027 × 411 000).

Sensitivity Analysis

In the preceding calculations, only one type of error has been acknowledged: that in the trawl survey biomass indices. This is the only source of the uncertainty expressed in the probability distribution estimated for B_0. To evaluate the effects of uncertainty in the model parameters of Table 1, a sensitivity analysis was carried out.

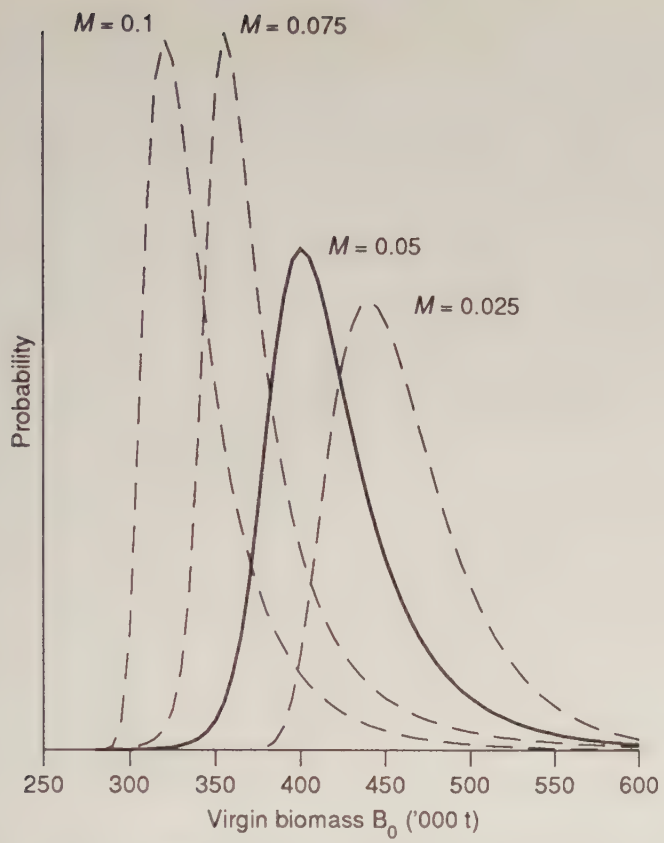

FIG. 2. Effect of different assumed values of natural mortality, M, on the estimated probability distribution function for virgin biomass, B_0. $M = 0.05$ is considered the most likely value.

The calculation of biomass and yield estimates was repeated eight times. For each repetition, the value of one (or a pair) of the parameters in Table 1 was changed slightly to see what effect that had on the estimates.

The results of the sensitivity analysis (Table 3) lead to two conclusions. First, there is greater certainty about biomass estimates than about yield because changes in the model parameters affect the latter much more than the former. Second, the greatest uncertainty derives from the natural mortality, M. Uncertainty in the growth parameters or ages at maturity, and entry to the fishery, are unlikely to change the yield estimate by more than 10%. Because fish born when the fishery started in 1978–79 will not enter the fishery until after the turn of the century, the apparently large effects of uncertainty in the steepness parameter are not relevant within the time frame of the risk analysis.

As a result of the sensitivity analysis, the biomass and yield calculations were repeated for a range of values of M (Table 4; Fig. 2).

Risk Analysis

The aim of the risk analysis was to evaluate the proposed strategy of reducing the TAC to the target value of 7500 t at a rate of 5000 $t \cdot yr^{-1}$ and to compare this strategy with alternative rates of reduction of 3000, 7000, 9000, and 12 000 $t \cdot yr^{-1}$ (Table 5).

Risk was expressed as the probability that the fishery would collapse within 5 yr. The fishery would be said to have collapsed if the TAC were not catchable with an instantaneous fishing mortality, $F < 1 \cdot yr^{-1}$. (This is equivalent to saying that the fishery has collapsed when the TAC is greater than two thirds of the beginning-of-season fishable biomass.)

Two sources of uncertainty were included in the Monte Carlo simulations used to estimate risk: uncertainty in B_0 and uncertainty in future recruitment.

The procedure for the simulation was as follows.

(1) A value of the virgin biomass, B_0, was chosen at random from the probability distribution estimated above.

(2) Using the population model in Appendix I, the fishery was simulated from this value of B_0 up to the present (i.e. up to and including the 1988–89 season) using the catches in Table 2 and deterministic recruitment.

(3) Simulations then continued, with random recruitment, up to and including the 1994–95 season using the TAC values from the appropriate row in Table 5. The catch for each year was set equal to either 1.3 times the TAC (in keeping with the assumed overrun in Table 2) or the catch obtained from applying a fishing mortality $F = 1$, whichever was the lesser.

(4) Steps 1–3 were repeated 200 times. The probability of collapse was calculated as the proportion of these 200 runs in which the fishery collapsed.

To increase comparability between simulation runs with different assumptions, the same random number sequence was used for each run.

The risk of collapse is strongly dependent on both the rate of TAC reduction and the assumed catch overrun in future seasons (Fig. 3A). The risk was, however, relatively insensitive to variation in the assumed value of c (CV of the trawl surveys). Recalculating the risk using the upper bound of $c = 19\%$, calculated above, made little difference (Fig. 3B).

Changes in M also had a relatively small effect on the risk of collapse (Fig. 3C) — small, that is, compared with the large effect such changes had on biomass and yield estimates (Table 4; Fig. 2). Estimated risks tended to be lowest for the "most likely" value of $M = 0.05$.

It is of interest to ask how much of the estimated risk is associated with uncertainty in B_0 and how much derives from random recruitment. Figure 3D shows the result of repeating the risk analysis with deterministic recruitment. The contribution of uncertainty in B_0 to the estimated risk decreases with

TABLE 5. TAC levels for the period 1989–90 to 1994–95 under five different rates of TAC reductions.

Rate of TAC reduction ($t \cdot yr^{-1}$)	Fishing year					
	1989–90	1990–91	1991–92	1992–93	1993–94	1994–95
3 000	28 637	25 637	22 637	19 637	16 637	13 637
5 000	28 637	23 637	18 637	13 637	8 637	7 500
7 000	28 637	21 637	14 637	7 637	7 500	7 500
9 000	28 637	19 637	10 637	7 500	7 500	7 500
12 000	28 637	16 637	7 500	7 500	7 500	7 500

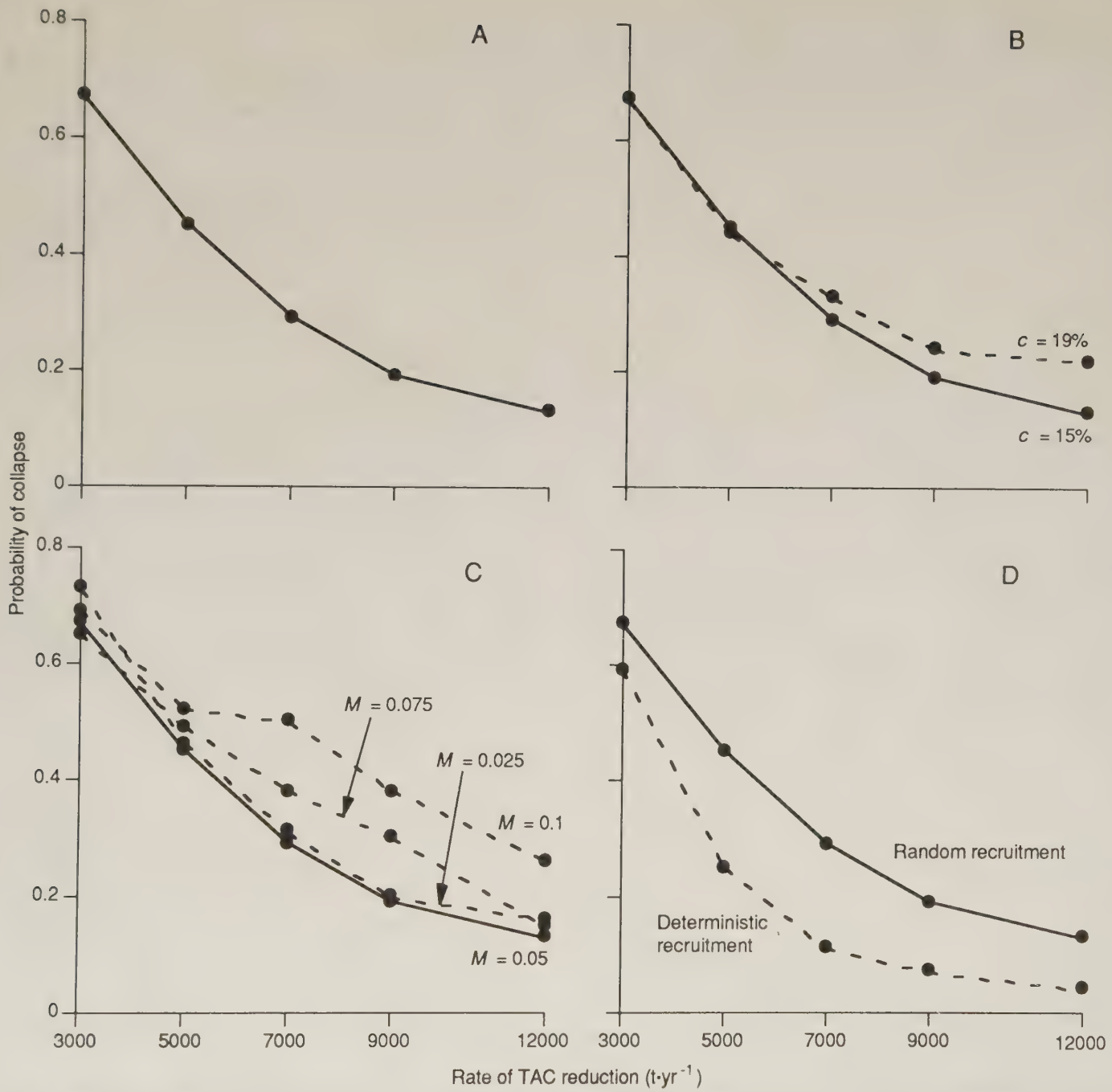

FIG. 3. Results of risk analysis. (A) Probability of fishery collapse within 5 yr if the TAC is stepped down to 7500 t at the rates shown; (B) effect on risk analysis of varying c, the CV of the trawl survey indices; (C) effect on risk analysis of varying M, the natural mortality; (D) effect on risk analysis of random recruitment. The solid lines in Fig. 3B–3D are the same as in Fig. 3A.

increasing rate of TAC reduction: from 88% (0.59/0.67) at 3000 t·yr^{-1} to 31% (0.04/0.13) at 12 000 t·yr^{-1}.

The risks shown in Fig. 3 only cover the 5-yr period up to October 1995. An examination of the biomass at the end of those simulation runs where the fishery did not collapse shows that there is a substantial probability that the biomass would still be dangerously low. For example, with the TAC reduced at 5000 t·yr^{-1} the probability that the October 1995 biomass is less than 20% of the virgin level is 0.73 (Fig. 4).

Figure 5 suggests that if the fishery is going to collapse within the next 5 yr, then it is likely to collapse sooner rather than later. If the TAC is reduced at 5000 t·yr^{-1}, then the most likely years of collapse are 1990–91 and 1991–92. For all the rates of TAC reduction considered, collapse is more likely before October 1992 than after.

Discussion

The advice to managers contained in Fig. 3A is valuable because it converts the uncertainty inherent in scientific knowledge about the status of the fish population into estimates of biological risk. The biological risk may then be considered by the fishery managers along with the economic and political risks. By contrast, the types of analyses previously used in New Zealand stock assessments would have lead to graphs such as Fig. 6, which, being completely deterministic, does little to

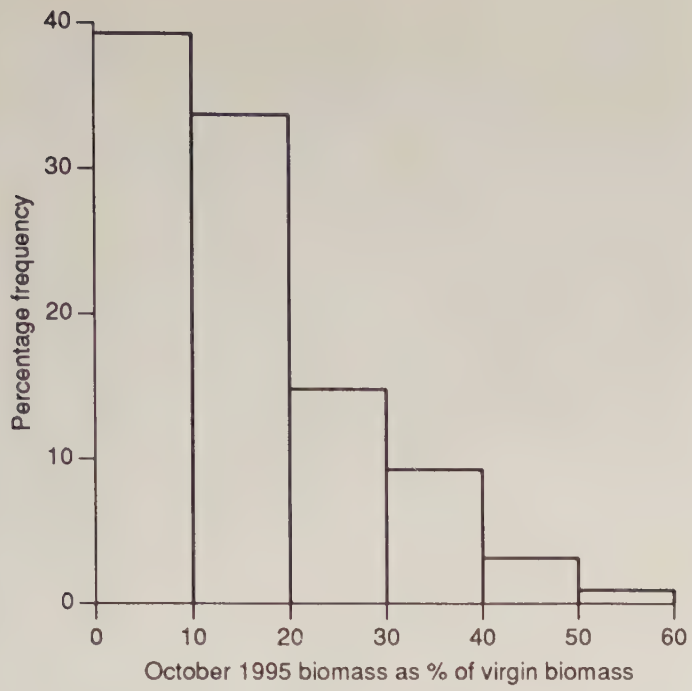

FIG. 4. Histogram of final biomass (as a percentage of the virgin biomass) for those simulation runs for which the fishery does not collapse; TAC reduced at 5000 $t \cdot yr^{-1}$.

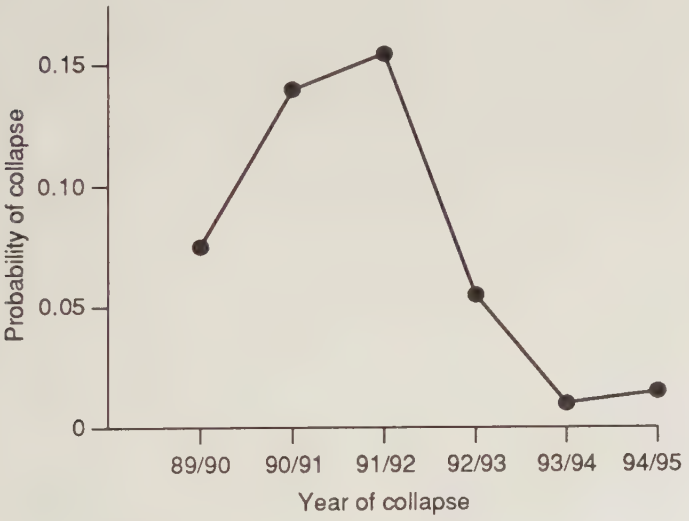

FIG. 5. Probability of collapse, by year; TAC reduced at 5000 $t \cdot yr^{-1}$.

demonstrate the dangerous position the fishery appears to be in.

A word of caution about the above results is appropriate. Although the probabilities of collapse shown in Fig. 3 are the best available, they should not be taken too literally. They indicate, given the assumed life history parameters and the catch history, the probability that a simple age-structured model of the fishery will collapse under different rates of TAC reduction. Fortunately, these results are not sensitive to changes in the parameters for which there is most uncertainty. What is not known is how well the model is able to predict the behaviour of the fish population. It may be that the true risks are substantially different from those given above or that another year's data will change our view of the state of the stock. However, the true risk of fishery collapse is just as likely to be greater

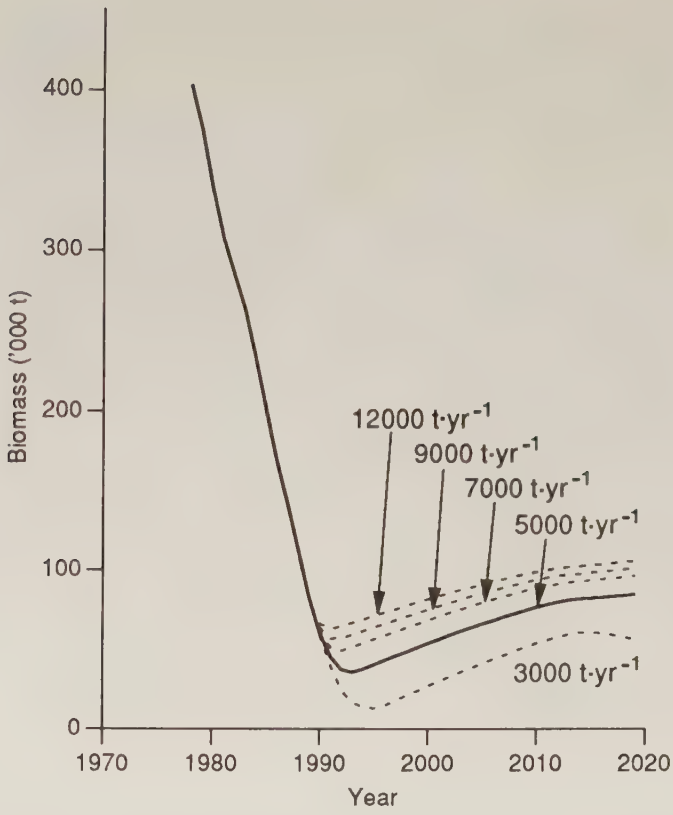

FIG. 6. Alternative presentation of management advice for orange roughy. The graph shows the "best guess" biomass projections under the five TAC reduction strategies, assuming $M = 0.05$. To convey some measure of uncertainty, it would be necessary to produce several versions of this graph with different possible values of B_0 and M. There is no simple way to include uncertainty about future recruitment to the fishery.

than the risks estimated here as it is to be less than them. Thus, it would seem prudent for managers to act as if the true risks are as estimated here.

In the remainder of this paper, I attempt to place the risk analysis technique presented above in the context of previously published methods and discuss how it may be generalized.

Other Risk Analyses

The approach to risk analysis used here is similar to those given by Linder et al. (1987) and Hall et al. (1988). The former used an event tree approach (rather than the Monte Carlo simulations used here) to calculate the probabilities of stock recovery under two levels of harvesting in the Georges Bank haddock (*Melanogrammus aeglefinus*) stock and Gulf of Mexico menhaden (*Brevoortia patronus*). This involved converting continuous ranges in population and recruitment abundance into discrete levels (high, medium, and low), with associated probabilities, and then calculating transition probabilities (e.g. the probabilities that high stock abundance in one year combined with a given level of harvesting and medium recruitment in the following year will lead to high, medium, or low stock abundance).

Hall et al. (1988) used Monte Carlo simulations to consider the more general problem of evaluating different harvesting policies (e.g. constant fishing mortality versus constant escapement) for Pacific herring (*Clupea harengus pallasi*). Rather than looking at a single measure of risk, they evaluated the policies

according to five measures: mean, variance, and CV of catch, the probability that the biomass would fall below a critical level, and the probability that no fishing would be allowed (possible under a constant escapement policy). Their second, third, and fifth measures could be seen as reflecting risk to the fishing industry, and the fourth measure as representing biological risk.

A related paper is that of Swartzman et al. (1987) who analyzed management approaches for Pacific hake (*Merluccius productus*). They used a more complex population model in which the parameters describing the state of the population at a given time include the variance and covariance of age-class abundance. This means that a management option may be evaluated with one run of the model, rather than several hundred runs as used in Monte Cario simulations. However, this is only possible with a very well-studied population for which such detailed parameters are estimable. Rather than estimating the risk associated with different management strategies, they imposed a minimum biomass constraint in order to protect the population and then looked to see which strategy performed best within that constraint.

Mendelssohn (1979) took a theoretical approach to the question of risk. Modelling a fishery as a Markov process, and formally defining a management policy as a set of decision rules, he investigated conditions under which there will exist policies that minimize risk.

Generalizations of the Risk Analysis

To generalize the approach to risk analysis taken in this paper, we may define risk as the probability of "something bad" occurring within a given time period. This definition provokes four questions: (1) "What is the best definition of 'something bad'?," (2) "What is an appropriate time period?," (3) "How should risk be calculated?," and (4) "What is an acceptable level of risk?"

For orange roughy, "something bad" was defined as the TAC not being caught with a fishing mortality $F < 1$ or, equivalently, the TAC being greater than about two thirds of the fishable biomass. This may seem harsh when a safe long-term level of exploitation for this fishery has been estimated as about 7% of the fishable biomass (author's unpublished data). An alternative and more conservative definition would be that "something bad" is the biomass falling below some threshold level, say 20% of B_0, as suggested by Beddington and Cook (1983). However, the orange roughy risk analysis was aimed at fishery managers and fishers. For this audience the possibility (or threat!) that the TAC may not be caught seemed a more immediate and compelling danger than crossing some arbitrary biomass threshold. Also, according to the above estimates, the biomass was expected to fall below 20% B_0 within a few months of the time of the analysis. In situations where the risk is not so immediate, a more conservative definition may be appropriate.

Similar comments are applicable to the question of an appropriate time period for the risk analysis. It is clear that the time period used above (5 yr) was not sufficiently long to encompass all the risk associated with the management strategies evaluated (Fig. 4). However, the analysis did show that the major risk of collapse was within this time period; and 5 yr is a natural period for the strategies considered because it takes about that much time to reduce the TAC to the target level. Also, an immediate risk to the fishery will only be communicated to managers and fishers by using a relatively short time period. Too short a time period would be counterproductive, as it would tend to delay the time at which it became apparent that TAC reductions were inevitable, and thus require a more rapid reduction, causing greater dislocation in the fishing industry.

The method of calculating risk will almost always, in the context of fishery management, be by Monte Carlo simulations. These provide a natural way of translating uncertainty — about the state of a fishery and the biology of the fish — into a measure of the risk associated with various alternative management strategies. In the orange roughy example, if calculated risk had been more dependent on the natural mortality parameter, M, a reasonable approach would have been to devise a subjective probability distribution for M and select a value from this distribution at step 1 of each simulation run. I have discussed above two alternative approaches (Linder et al. 1987; Swartzman et al. 1987) which avoid Monte Carlo methods and so calculate risks analytically. The latter seems to me desirable (when sufficiently detailed data are available); the former involves approximations which are hard to justify unless available computing power is very limited.

The last of my four questions concerns an acceptable level of risk. In the orange roughy application described above, this question is a matter for the fishery manager rather than the scientist and so is beyond the scope of this paper. However, it should be pointed out that the question cannot be answered without an evaluation of the relative difficulties experienced by the fishing industry in relation to the different rates of TAC reduction. In more abstract applications of risk analysis, it will be necessary to define an acceptable level of risk. For example, the definition of MCY (see Yield Estimation, above) includes the phrase "with an acceptable level of risk." To make this definition rigorous, this level would need to be quantified.

There are alternatives to the method presented here for estimating a probability distribution for B_0. For example, a bootstrap procedure or Bayesian methodology could be used. The effect of these alternative methods on the risk estimator is currently under study and this work will be reported elsewhere.

One way of extending the orange roughy analysis would be to introduce economic data (on the cost of fishing, market value of orange roughy, etc.) into the model. This would allow us to express the uncertainty in terms of risk to the fishing industry (using net present value, for instance) and thus make the choice of management strategies on purely economic grounds. However, it is not a simple matter to find a good measure of risk to the industry (even if the economic data were available). Industry's perception of the risk associated with harvesting orange roughy will depend on a wide range of variables, such as the debt levels on vessels, alternative species available for harvesting, the impact of a change in quota on marketing commitments, and the need for a continuous product flow in processing factories. The perception would be different for different companies. Further, most fishery managers will wish to maximize the long-term yield from the fishery (for New Zealand managers, this is a legal requirement) and thus will not be able to ignore the biological risks. The task of balancing biological risk against dislocation to the industry is an example of the difficult multiple objective problem, of which Peterson and Smith (1982) have commented, "[it] is widely recognized, but very little has been done about it." I make no contribution to solving this problem. What I hope I have illustrated is how risk analysis can enhance the assessments made by scientists for fishery managers.

Acknowledgements

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Appendix I: Age-Structured Population Model

Two conventions are followed in this model. First, "biomass" means midyear biomass, i.e. the level after half the year's mortality has occurred. Similarly, numbers at age are midyear estimates. This is because the orange roughy trawl surveys were carried out at a time of year when it was reasonable to assume that about half the year's fishing had occurred. The second convention is that the words "recruit" and "recruitment" refer to entry to the population from the larval phase — arbitrarily set at age 1. The term "fishable" refers to fish that have entered the fishery.

The basic equation of this model shows how to calculate $N_{i,j}$, the number of j-yr-old fish in the population in year i, in terms of the previous year's numbers:

$$N_{i,j} = \begin{cases} R_i e^{-0.5M} & \text{for } j = 1 \\ N_{i-1,j-1} e^{-M} & \text{for } 1 < j < A_f \\ N_{i-1,j-1} e^{-0.5(M+Z_i)} & \text{for } j = A_f \\ N_{i-1,j-1} e^{-0.5(Z_{i-1}+Z_i)} & \text{for } A_f < j < A_{\max} \\ (N_{i-1,A_{\max}} + N_{i-1,A_{\max}-1})\, e^{-0.5(Z_{i-1}+Z_i)} & \text{for } j = A_{\max} \end{cases}$$

where A_f is the age at entry to the fishery, R is the number of recruits, M and F are the instantaneous natural and fishing mortalities, respectively, and $Z = M + F$ is the total mortality. M is assumed constant for all years and all age classes; F and Z vary from year to year, but not between fishable age classes.

The age group with $j = A_{\max}$ is called a plus group: it contains all fish of age $A_{\max}$ and older. The value chosen for $A_{\max}$ has little effect on calculations as long as it is large enough that the weight of fish of age $A_{\max}$ is close to the asymptotic weight. For orange roughy, I used $A_{\max} = 70$ yr. The fishable biomass in year i is given by

$$B_i = \Sigma_f N_{i,j} w_j$$

where w_j is the mean weight of fish of age j, calculated from the von Bertalanffy mean length at age equation and the mean weight at length equation (Table 1), and Σ_f means summation over all fishable year classes, i.e. from $j = A_f$ to $j = A_{\max}$. Similarly, Σ_m means summation over all mature age-classes. Beginning- or end-of-year biomass may be calculated by multiplying B_i by $e^{0.5Z_i}$ or $e^{-0.5Z_i}$, respectively.

The catch in year i, C_i, is given by

$$C_i = B_i e^{0.5Z_i}(1 - e^{-Z_i})(F_i/Z_i).$$

For the calculations in this paper the C_i were known but the F_i were not, so the latter were calculated from the former using this equation and a simple search algorithm.

The population model has two forms: random and deterministic. In the deterministic form, we assume that the virgin population has a stable age distribution. This means that the virgin number at age j, $N_{0,j}$, is given by

$$N_{0,j} = \begin{cases} R_0 e^{-M(j-0.5)} & \text{for } j < A_{\max} \\ R_0 e^{-M(A_{\max}-0.5)}/(1 - e^{-M}) & \text{for } j = A_{\max}. \end{cases}$$

It will sometimes be convenient to define $n_{0,j}$ as the number of fish of age j per recruit and write $N_{0,j} = R_0 n_{0,j}$.

The usual form of the Beverton and Holt stock–recruit relationship for deterministic recruitment is

$$R_i = A_{i-1}/(\alpha + \beta A_{i-1})$$

where A is the biomass of mature fish and α and β are parameters that have no biological meaning. However, the assumption of a stable age distribution for the virgin population allows us to express these parameters in terms of the virgin recruitment, R_0, and a parameter h, called the "steepness" of the stock–recruit relationship. The latter parameter is related to the steepness of the early part of the stock–recruit curve: specifically, the number of recruits when the mature biomass is reduced to 20% of its virgin level is hR_0. Thus, we have

$$R_0 = A_0/(\alpha + \beta A_0)$$

$$hR_0 = 0.2A_0/(\alpha + 0.2\beta A_0)$$

and, solving these equations for α and β, we get

$$\alpha = A_0(1 - h)/(4hR_0) = (\Sigma_m n_{0,j} w_j) e^{-0.5M}(1 - h)/(4h)$$

$$\beta = (5h - 1)/(4hR_0).$$

In this paper, calculations with the model always start with a virgin population. Given values for the parameters in Table 1, only one further parameter is required, viz the virgin fishable biomass, B_0. Since

$$B_0 = (\Sigma_f N_{0,j} w_j) = R_0(\Sigma_f n_{0,j} w_j),$$

R_0 may be calculated as

$$R_0 = B_0/(\Sigma_f n_{0,j} w_j).$$

In the case where the recruitment, R_i, is random, it is generated as a lognormal random variable with mean value given by the Beverton and Holt stock–recruit equation (above). The other parameter describing this random variable is σ_R, the standard deviation of the natural log of R_i.

Appendix II: Fitting of Johnson's S_U Distribution

If Z is a normal random variable with zero mean and standard deviation 1, then

$$B = \xi + \lambda\sinh[(Z - \gamma)/\delta]$$

is distributed according to Johnson's S_U distribution (Johnson and Kotz 1970, p. 23). The cumulative distribution function (cdf) for B is

$$G(b) = F(\gamma + \delta\sinh^{-1}[(b - \xi)/\lambda])$$

where F is the cdf of Z.

Because the S_U distribution is very flexible, it is reasonable to assume that there exist values of ξ, λ, γ, and δ such that the probability distribution for the virgin biomass, B_0, of the Chatham Rise orange roughy population is well approximated by the S_U distribution with these parameters.

For each of b_i, $i = 1,\ldots,n$ (n trial values of B_0), we observe the estimated probabilities p_i, $i = 1,\ldots,n$. The p_i are distributed according to a binomial distribution with parameters m_i and $G(b_i)$ (where m_i is the number of simulations carried out with trial B_0 value b_i). The log-likelihood for the observed p_i may be shown to be (ignoring constant terms)

$$m_i p_i \log[G(b_i)] + m_i(1 - p_i)\log[1 - G(b_i)].$$

A standard minimization package (Mittertreiner and Schnute 1985) was used to find the values of ξ, λ, γ, and δ that maximized this log-likelihood.

Optimal Harvest Rates for Mixed Stocks of Natural and Hatchery Fish

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Kope, R. G. 1992. Optimal harvest rates for mixed stocks of natural and hatchery fish. Can. J. Fish. Aquat. Sci. 49: 931–938.

Optimal harvest rates were computed using dynamic programming for mixed-stock fisheries exploiting two stocks of either natural or hatchery origin. Natural stocks were described by a Ricker spawner–recruit relationship and hatchery stocks were described by a rectilinear spawner–recruit relationship. Harvest rates were optimized for both risk-neutral and risk-averse utility functions. For two natural stocks with low productivities, optimal harvest rates generally appeared to favor the stronger stock for a risk-neutral utility function and the weaker stock for a risk-averse utility function. For both utility functions, optimal harvest policy became less sensitive to relative stock strength as the productivity of the stocks increased. When at least one of the stocks was of hatchery origin, optimal harvest policy favored the weaker stock using either utility function.

En utilisant une programmation dynamique, l'auteur a calculé des taux d'exploitation optimum dans le cas de pêches axées sur deux stocks, soit d'origine sauvage ou d'élevage. Il a décrit les stocks d'origine sauvage à l'aide d'une relation géniteur–recrue de Ricker et les stocks d'élevage, d'une relation rectilinéaire géniteur–recrue. Il a ensuite optimalisé les taux d'exploitation des fonctions d'utilité à risque neutre et à aversion pour le risque. Dans le cas de deux stocks naturels à faible taux de productivité, les taux d'exploitation optimum semblaient généralement favoriser le plus important stock pour ce qui est de la fonction d'utilité à risque neutre, et le stock le moins abondant pour ce qui est de la fonction d'utilité à aversion pour le risque. Dans le cas des deux fonctions d'utilité, une politique d'exploitation optimum a moins réagi à l'abondance relative d'un stock lorsque la productivité des stocks augmentait. Lorsque au moins un des stocks était issu de l'élevage, une politique d'exploitation optimum favorisait le stock moins abondant pour ce qui est des deux types de fonctions d'utilité.

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Mixed-stock fisheries present some complex and persistent problems in the management of renewable resources. Problems associated with identification of spawner–recruit relationships (Ricker 1973; Hilborn 1985a), loss of stock diversity (Ricker 1958; Paulik et al. 1967), and optimal harvest from mixed-stock fisheries (Ricker 1958; Paulik et al. 1967; Hilborn 1976, 1985b; Collie et al. 1990) have all been investigated using Pacific salmon as examples. Pacific salmon stocks are particularly suited for investigating mixed-stock problems. Because of their fidelity to natal streams, individual salmon stocks can be clearly identified on the spawning grounds, and it is possible to monitor individual stocks over time. Virtually all salmon fisheries harvest a mixture of stocks that can differ widely in size, productivity, and variability. Natural salmon production is also supplemented by production from hatcheries that have been constructed to mitigate the impacts of water development projects and to enhance fisheries. Hatcheries are typically more productive than natural stocks in the sense that they produce more recruits per spawner than do naturally spawning stocks. The proliferation of hatcheries has prompted concerns that fisheries supported by hatchery stocks may severely deplete less productive natural stocks (Wright 1981).

The particular problem addressed here is that of optimal harvest of mixed stocks by a common fishery. Ricker (1958) presented a graphical solution to this problem and showed that maximum equilibrium yield for mixed-stock fisheries could, in some cases, be obtained at harvest levels that would drive some component stocks to extinction. Paulik et al. (1967) derived a general solution demonstrating that maximum sustainable yields from mixed-stock fisheries may entail elimination of less productive stocks, and declines in the spatial heterogeneity of Pacific salmon stocks have been observed coincident with increasing fishing mortality rates (Walters and Cahoon 1985).

Hilborn (1976), using dynamic programming, investigated optimal harvesting of two stocks by a common fishery. This technique allows for inclusion of uncertainty about future production and relaxation of the assumption of equilibrium conditions. Hilborn (1976) solved for optimal harvest rates for specific combinations of stock abundances, demonstrated that optimal harvest rates depend on the relative abundance of the component stocks, and concluded that, in general, mixed-stock fisheries should be harvested more heavily when the ratio of stocks differs from 1:1. Optimal harvest policies determined by dynamic programming assume that managers have the ability to forecast the abundance of individual stocks that contribute to a mixed-stock fishery. Hilborn (1985b) later pointed out that the requirement of individual forecasts for the component stocks was unrealistic and sought optimal management strategies from a class of strategies that does not require forecasting of individual component stocks.

In this paper, I use dynamic programming to evaluate harvest strategies that optimize risk-neutral (Hilborn 1976; Mendelssohn 1982; Deriso 1985) and risk-averse (Mendelssohn 1982; Deriso 1985; Walters and Ludwig 1987; Parma 1990) utility functions for a single fishery harvesting two stocks.

The spawner–recruit relationships (SRRs) used to model stock dynamics are chosen to represent naturally produced fish and hatchery-produced fish. I also reexamine optimal harvest policies for a fishery harvesting two natural stocks using a finer grid for stock sizes and a broader range of parameter values than were used by Hilborn (1976). I show that the functional forms of the SRRs and the utility function can profoundly affect optimal harvest strategies. Although I examine only a few specific cases for two-stock fisheries, the results suggest some general conclusions that may be applicable to cases where individual stock forecasts are not possible and to cases involving more than two stocks.

Methods

Following Hilborn (1976), I used a Ricker SSR to describe recruitment to a natural population. The particular form used was

$$(1)\quad R_{t+1} = S_t \exp\{\alpha[1 - (S_t/\beta)] + \epsilon_t\}$$

where R_{t+1} is the number of recruits resulting from S_t spawners, α is a productivity parameter (specifically, the natural log of the number of recruits produced by each spawner in the absence of density-dependent effects), β is the equilibrium stock size (i.e. the level of spawning escapement where each spawner exactly replaces itself in the absence of fishing mortality), and ϵ_t are independently distributed normal random variables with zero mean and variance σ^2. Hatchery stocks were assumed to have a rectilinear SRR of the form

$$(2)\quad R_{t+1} = \min(\alpha S_t, \beta) \exp(\epsilon_t).$$

This functional form presumes that recruitment of hatchery fish is, on average, proportional to the number of fish spawned at the hatchery, with no density dependence when the abundance of spawners is less than the hatchery capacity. If the number of spawners exceeds the hatchery capacity, the excess spawners have no effect on recruitment.

Omitting age structure and natural mortality, recruitment equals preharvest abundance, simplifying both the population model and the optimization problem. The description of population dynamics is completed by

$$(3)\quad S_t = R_t - C_t$$

where C_t is catch from generation t in numbers of fish and harvest rate is defined as

$$(4)\quad h_t = C_t / R_t.$$

I computed approximately optimal harvest policies using dynamic programming following the procedure outlined in Hilborn (1976), but using a finer discretization. Where Hilborn (1976) used 20 abundance levels, 18 harvest rates, and 10 stochastic outcomes, I used 80 abundance levels, 40 harvest rates, and 20 stochastic outcomes with $\sigma = 0.5$. This finer discretization increases the resolution of the approximate solutions and reveals details that are not readily apparent on the coarser grid.

Hilborn (1976) observed that for two stocks governed by equation (1), there are five nonredundant possibilities for the stock–recruitment dynamics: (1) $\alpha_1 = \alpha_2$, $\beta_1 = \beta_2$, (2) $\alpha_1 > \alpha_2$, $\beta_1 = \beta_2$, (3) $\alpha_1 = \alpha_2$, $\beta_1 > \beta_2$, (4) $\alpha_1 > \alpha_2$, $\beta_1 > \beta_2$, and (5) $\alpha_1 > \alpha_2$, $\beta_1 < \beta_2$. Other possible relationships involve simply interchanging stock 1 and stock 2. However, in addition to depending on the relative magnitudes of the stock productivities (i.e. the ratio of α_1 to α_2), optimal harvest strategies also depend on the absolute magnitude of the stock productivities with every unique combination of productivities producing a unique solution. In contrast, the equilibrium stock levels serve merely to scale the spawner–recruit curves, and a constant ratio of β_1 to β_2 will always produce the same solution relative to equilibrium stock sizes regardless of the absolute magnitude of the equilibrium stock sizes. Because it is impossible to examine all potential combinations of α and β, I considered only a limited number of cases. I examined optimal harvest rates for stocks of low ($\alpha = 0.5$), moderate ($\alpha = 1.5$), and high ($\alpha = 2.5$) productivities (Fig. 1) and with either identical βs or βs that differed by a factor of 2. Solutions were obtained for both risk-neutral and risk-averse objective functions by maximizing either the sum of expected catches over the planning horizon or the sum of the natural logarithms of expected catches over the planning horizon. Both of these utility functions are maximized in the last time period by harvesting the entire stock. As we work backward in time, the expected contribution of future catches to the objective function increases and lower harvest rates maximize the objective functions. Working backward from the last time period, optimal harvest strategies usually stabilize within 10 yr. In all cases presented here, harvest rates were optimized by calculating backward over a 20-yr planning horizon.

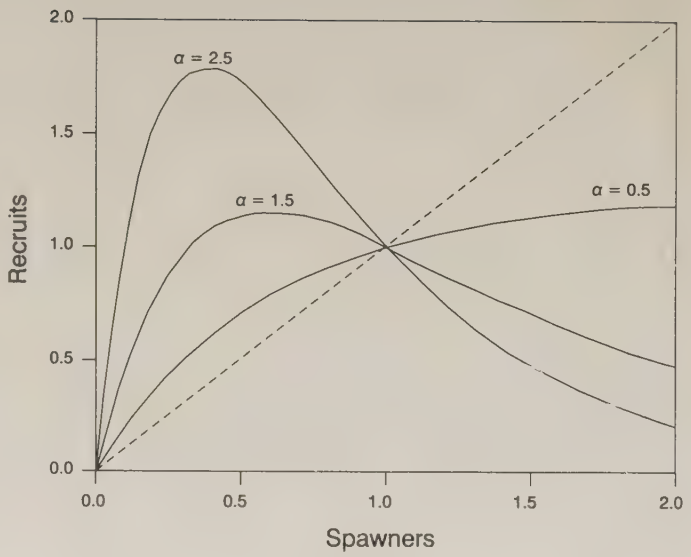

FIG. 1. Spawner–recruit curves used for the "natural" stocks in the dynamic programming estimation of optimal harvest rates for low-productivity stocks ($\alpha = 0.5$), moderate-productivity stocks ($\alpha = 1.5$), and high-productivity stocks ($\alpha = 2.5$). Unexploited equilibrium stock size (β) is equal to 1.0.

Results

Two Natural Stocks

For each possible combination of stock sizes, the dynamic programming algorithm computes the harvest rate that optimizes the objective function by maximizing the utility of the catch obtained in the present time period and the expectation of future catches from the spawning escapement. For a single

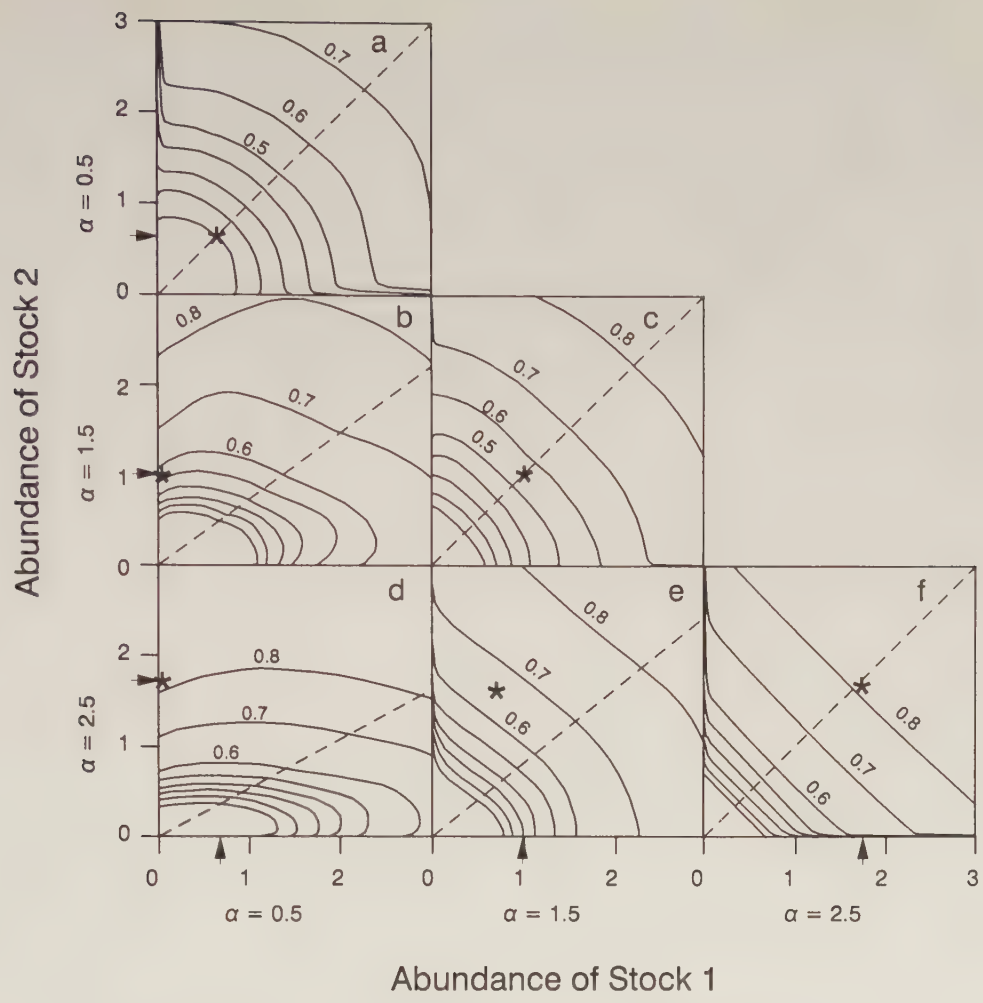

FIG. 2. Optimal risk-neutral harvest rate contours for stocks with identical unexploited stock size ($\beta = 1.0$). Individual stock MSY equilibria are indicated by arrows on the figure axes, and the joint MSY equilibria are indicated by asterisks. The broken line indicates isoharvest proportions where identical harvest rates are optimal for both individual stocks.

stock governed by the Ricker SRR the harvest policy that optimizes the linear (risk-neutral) utility function is to harvest that portion of the recruitment (abundance) that is in excess of some optimal spawning escapement. If abundance falls below this optimal escapement, then no harvest is taken and the stock is allowed to rebuild (Walters 1975; Parma 1990). Associated with each nonzero harvest rate is a single abundance level for which that harvest rate is optimal. In a two-stock fishery, the combinations of component stock abundances that share the same individual optimal harvest rates describe a path through the stock abundance plane. Along this trajectory, which I will call the "isoharvest" line, the optimal harvest rate for the mixed-stock fishery is also optimal for each component stock individually (Fig. 2). For combinations of stock abundances that lie above the isoharvest line, the harvest rates that would be optimal for the stock plotted on the ordinate (stock 2) are higher than the optimal harvest rates for the stock on the abscissa (stock 1). Thus, above the isoharvest line, stock 2 is, in a sense, the stronger stock and stock 1 the weaker stock. For any stock abundance combination that does not lie on this isoharvest line, the optimal harvest rate in the mixed-stock fishery will be intermediate between the harvest rates that are optimal for the two component stocks at their respective abundances.

When the two component stocks are governed by identical SRRs the isoharvest line is a straight line with slope 1.0 (Fig. 2a, 2c, and 2f). Stock combinations producing constant levels of total abundance describe straight lines in the stock abundance plane with slopes of -1.0. If optimal harvest rates depend only on the overall abundance, and not on the stock composition, optimal harvest rate contours will coincide with these constant levels of total abundance. If contours of optimal harvest rates deviate from these straight lines with slope -1.0, the optimal harvest policy is stock dependent.

The dependence of optimal harvest policy on stock productivities is readily apparent (Fig. 2). Hilborn (1976) reported that the optimal harvest policy for two identical stocks was a constant escapement policy which would be represented by straight contours with a slope of -1. This is approximately true only when the stocks have high productivity (Fig. 2f). For low-productivity stocks (Fig. 2a), and to a lesser extent moderate-productivity stocks (Fig. 2c), the optimal harvest rate contours are concave. This means that for identical stocks with low productivities or moderate productivities, if the stock composition differs from the isoharvest ratio of 1:1, the optimal harvest rate is higher than it would be for the same total abundance partitioned equally among the two component

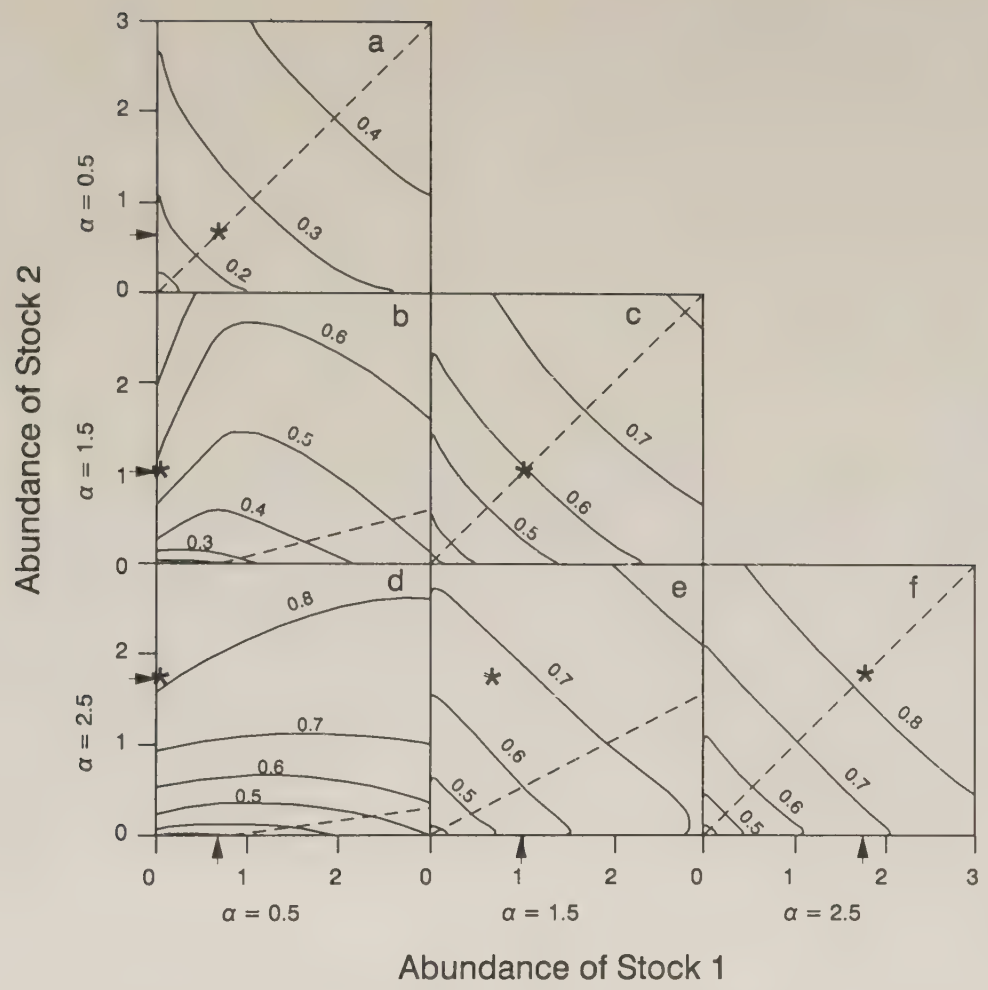

FIG. 3. Optimal risk-averse harvest rate contours for stocks with identical unexploited stock size ($\beta = 1.0$). Individual stock MSY equilibria are indicated by arrows on the figure axes, and the joint MSY equilibria are indicated by asterisks. The broken line indicates isoharvest proportions where identical harvest rates are optimal for both individual stocks.

stocks. For stocks with high productivities the optimal harvest rate contours are slightly convex, indicating that optimal harvest rates are slightly lower if abundance is not partitioned equally between the two component stocks, but the convexity is so slight that the optimal harvest policy could be considered stock indifferent for most reasonable abundance combinations.

While optimal harvest rates for all stock combinations are of interest, some stock combinations are far more likely to occur than others. Solutions presented here assume that future harvest rates will also be optimized, and continual management for optimal yields will tend to hold the stocks near the equilibrium levels that would support maximum sustainable yields in the absence of random variability (MSY equilibrium). If the low-productivity stock is harvested jointly with either the moderate- or high-productivity stocks, the low-productivity stock becomes extinct at MSY equilibrium (Fig. 2b and 2d). When MSY equilibrium supports only one stock, optimal harvest rates are understandably more sensitive to changes in the abundance of the stock that will remain at equilibrium than they are to changes in the stock destined for extinction. For the combination of moderate- and high-productivity stocks (Fig. 2e), MSY equilibrium results from a mixed-stock fishery and the optimal policy is nearly stock indifferent in the vicinity of this equilibrium. However, optimal harvest policy is more sensitive to the less productive stock if it is either at extremely low levels or at high levels relative to the more productive stock, reducing harvest rates if the less productive stock is in danger of extinction and increasing harvest rates if it becomes superabundant.

Optimal risk-averse harvest policy is less dependent on overall abundance, with higher harvest rates at low stock levels and lower harvest rates at high stock levels, than risk-neutral management (Fig. 3). This is consistent with the tendency for optimal risk-averse policies to approach constant harvest rates, producing less variability in catches. The decrease in variability of harvest rates produces a greater disparity in the isoharvest abundance ratios for stocks that differ in productivity (Fig. 3b, 3d, and 3e). Like optimal risk-neutral harvest policies, optimal risk-averse policies are less sensitive to stocks destined for extinction at the MSY equilibrium (Fig. 3b and 3d). In the remaining cases, where the MSY is obtained from a mixed-stock fishery, the deviations in optimal harvest rates associated with variation in stock composition appear to be the opposite of deviations in optimal risk-neutral harvest policy. For low-productivity stocks, optimal policy decreases harvest rates when the composition of stocks is inequitable, and the stock dependency of optimal harvest policy decreases as stock productivity increases.

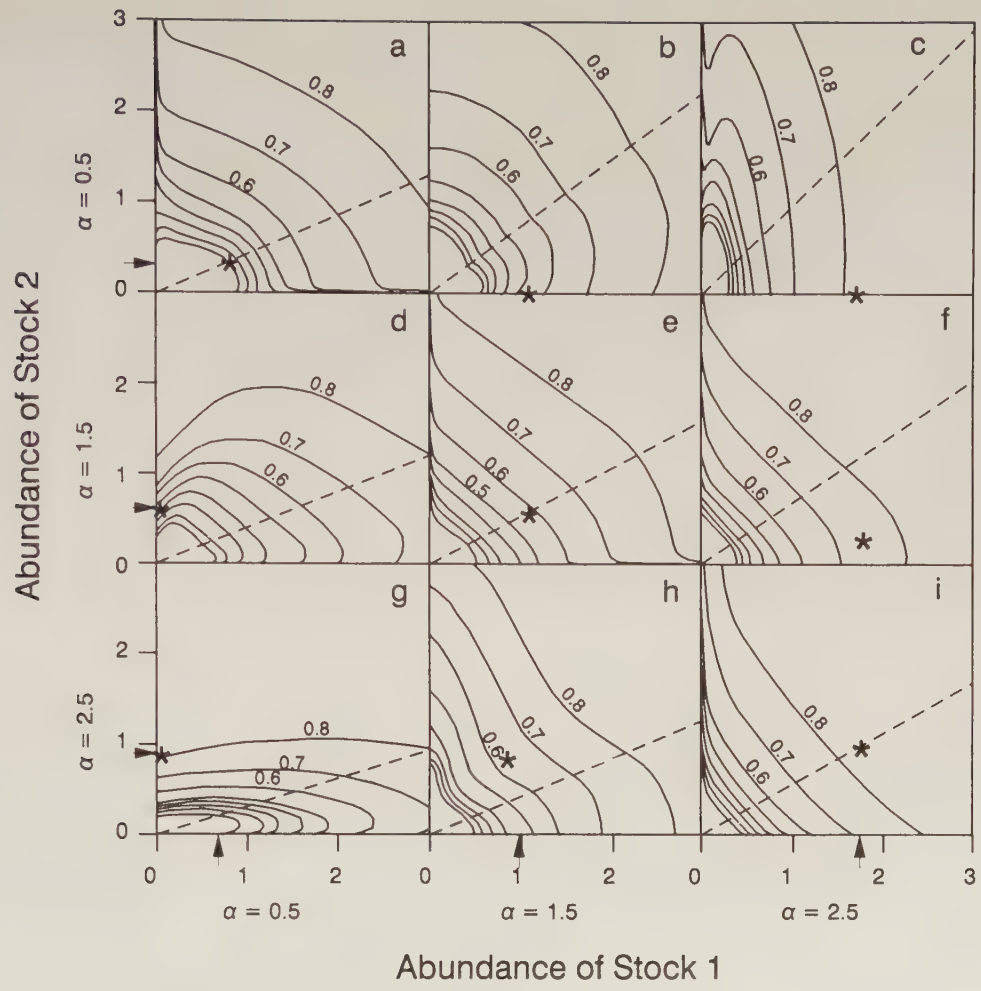

FIG. 4. Optimal risk-neutral harvest rate contours for stocks with different unexploited stock equilibria ($\beta_1 = 1.0$, $\beta_2 = 0.5$). Individual stock MSY equilibria are indicated by arrows on the figure axes, and the joint MSY equilibria are indicated by asterisks. The broken line indicates isoharvest proportions where identical harvest rates are optimal for both individual stocks.

For two natural stocks with different βs, optimal risk-neutral and risk-averse harvest policies are qualitatively similar to corresponding cases with identical βs (Fig. 4 and 5). In all cases, optimal harvest rates are less sensitive to stocks that will be extinct at the MSY equilibrium than to the stocks that will survive. Again, optimal risk-averse harvest policy is less sensitive to the relative abundance of the two component stocks at a given level of overall abundance than is risk-neutral management. Of particular interest is the case of a moderate-productivity stock with large β and a high-productivity stock with small β under risk-neutral management (Fig. 4h). With this combination of stocks, the expected contributions from the two component stocks to the combined yield are very similar. In the vicinity of the MSY equilibrium, there is a ridge in the optimal harvest rate contours. This depicts an optimal harvest strategy that decreases harvest rates when the ratio of stock abundances differs from the equilibrium ratio at MSY.

Hatchery and Natural Stocks

The optimal risk-neutral harvest strategies for mixed fisheries of hatchery and natural stocks differ dramatically from those involving two natural stocks (Fig. 6). An abrupt ridge occurs in the optimal harvest rate contours coincident with the isoharvest line, and optimal harvest rates decrease as the ratio of stocks departs from the isoharvest ratio. The MSY equilibrium entails far greater relative abundance of the hatchery stock than the isoharvest ratio. In all cases the MSY equilibrium occurs with both stocks present and the hatchery stock at its individual MSY abundance level, but bear in mind that the rectilinear SRR used for the hatchery stock ensures that the expected abundance of the hatchery stock is constant unless harvest rates are greater than the productivity of the hatchery stock. The MSY equilibrium for the natural stock is noticeably less than its individual MSY level only in the case where the unfished equilibrium for the hatchery stock is larger than that of the natural stock. In the region of the MSY equilibrium the optimal harvest rates are much more sensitive to changes in the natural stock than to changes in the hatchery stock.

Under risk-averse management, this prominent ridge in the optimal harvest rate contours is even more pronounced (Fig. 7). Again, optimal risk-averse harvest rates are less sensitive to changes in total abundance than are optimal risk-neutral harvest rates. However, unlike cases involving two natural stocks, optimal harvest rates are more sensitive to changes in the stock composition than are harvest rates under risk-neutral management. In the region of the MSY equilibrium, which is now even

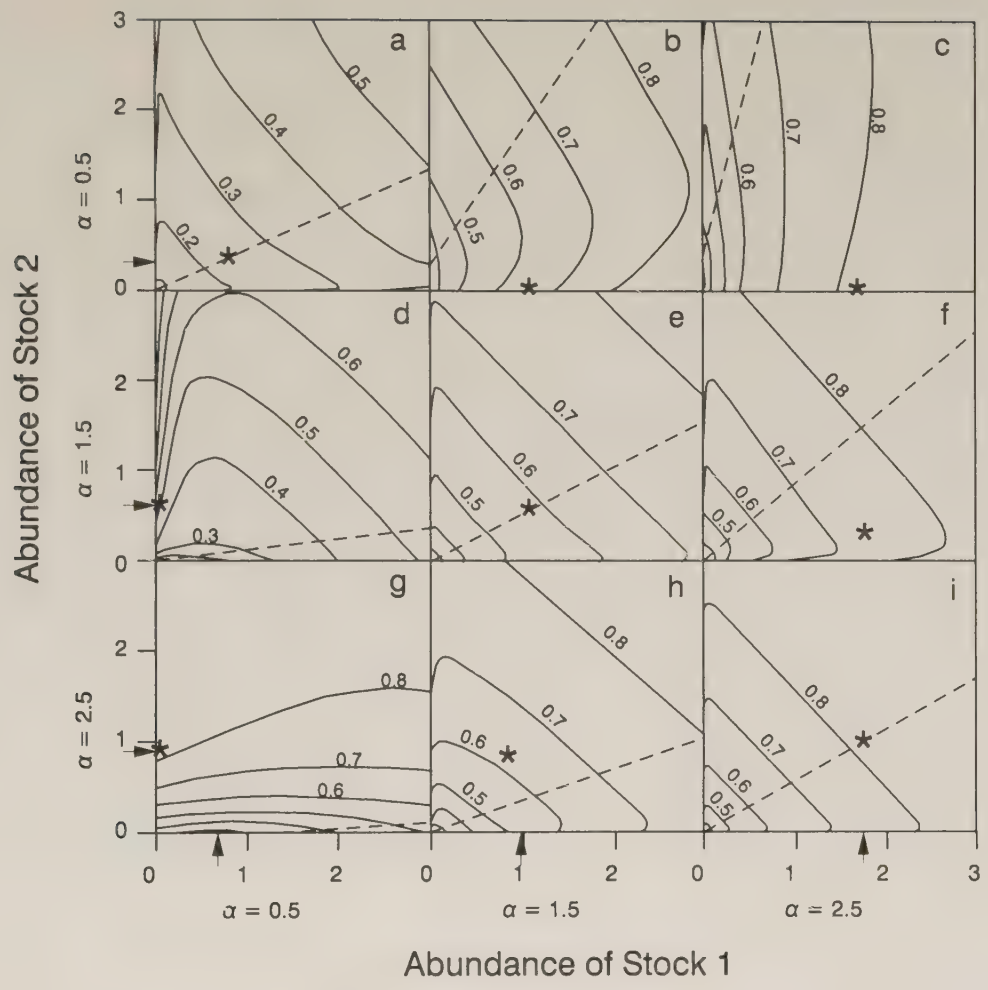

FIG. 5. Optimal risk-averse harvest rate contours for stocks with different unexploited stock equilibria ($\beta_1 = 1.0$, $\beta_2 = 0.5$). Individual stock MSY equilibria are indicated by arrows on the figure axes, and the joint MSY equilibria are indicated by asterisks. The broken line indicates isoharvest proportions where identical harvest rates are optimal for both individual stocks.

further from the isoharvest line, optimal harvest rate contours are vertical, indicating that optimal harvest policy is independent of changes in the hatchery stock.

The presence of the sharp ridge in the optimal harvest rate contours for mixed-stock fisheries results from the rectilinear SRR used for the hatchery stock. Above the isoharvest line the combinations of hatchery stock abundance and harvest rates that are optimal for the natural stock ensure that hatchery spawners will exceed hatchery capacity. Changes in the harvest of hatchery fish have no effect on future stock dynamics, only on harvest at the present time. At the same time, changes in the harvest of natural fish affect both the current harvest and future harvests. Consequently, optimal harvest rates are more sensitive to abundance of the natural stock than of the hatchery stock. Below the isoharvest line the hatchery stock is at sufficiently low abundance that harvest rates that are optimal for the natural stock will fail to provide enough hatchery spawners to fully utilize hatchery capacity. At this point, optimal harvest rates are driven by full utilization of hatchery capacity unless the disparity in stock sizes is so great that the cumulative loss from underharvesting the natural stock would exceed the loss from a one-time failure to fully utilize hatchery capacity. If we remove the abrupt transition in the hatchery SRR, by substituting a Beverton–Holt SRR for the rectilinear SRR, the ridge in the optimal harvest rate contours nearly disappears, as does much of the dependence on hatchery stocks (Fig. 8).

Discussion

Equilibrium analyses can place an upper limit on the average yield that can be expected from a mixed-stock fishery, and the component stocks that will still be around at equilibrium to contribute to this yield, but real fisheries are perpetually in a state of disequilibrium. Dynamic programming can approximate optimal harvest policies that embrace disequilibrium and uncertainty for specified SRRs, but the computational burden limits the size and complexity of problems that can be addressed. While the optimal harvest rates in any particular case depend on the SRRs chosen to describe stock dynamics and the specific parameter values used, a few general axioms can be inferred from the simulations presented here.

(1) For unproductive natural stocks, optimal risk-neutral harvest rates are more sensitive to changes in the stronger stock and optimal risk-averse harvest rates are more heavily influenced by the weaker stock.

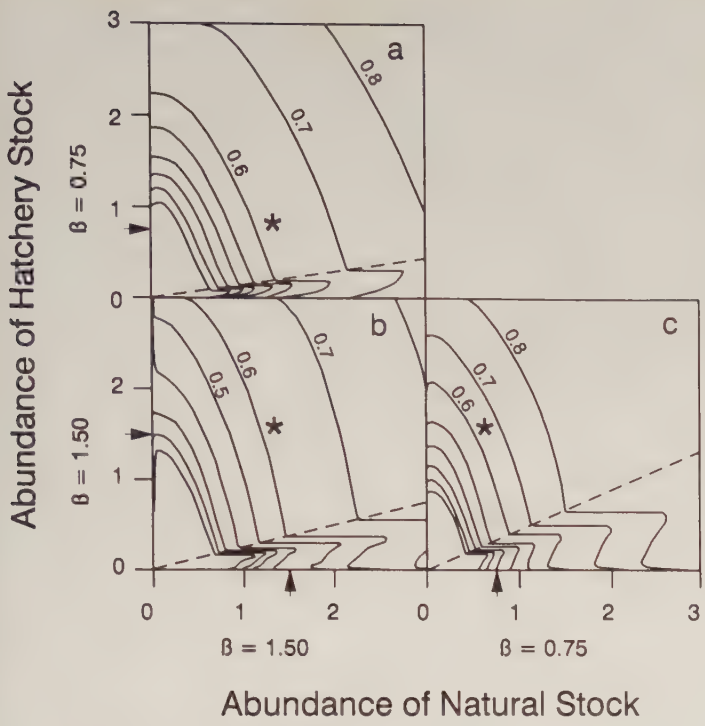

FIG. 6. Optimal risk-neutral harvest rate contours for stocks with different production functions. The natural stock is governed by a Ricker SRR with moderate productivity ($\alpha = 1.5$), and the hatchery stock is governed by a high-productivity ($\alpha = 2.5$) rectilinear SRR. Individual stock MSY equilibria are indicated by arrows on the figure axes, and the joint MSY equilibria are indicated by asterisks. Isoharvest proportions occur along the broken lines.

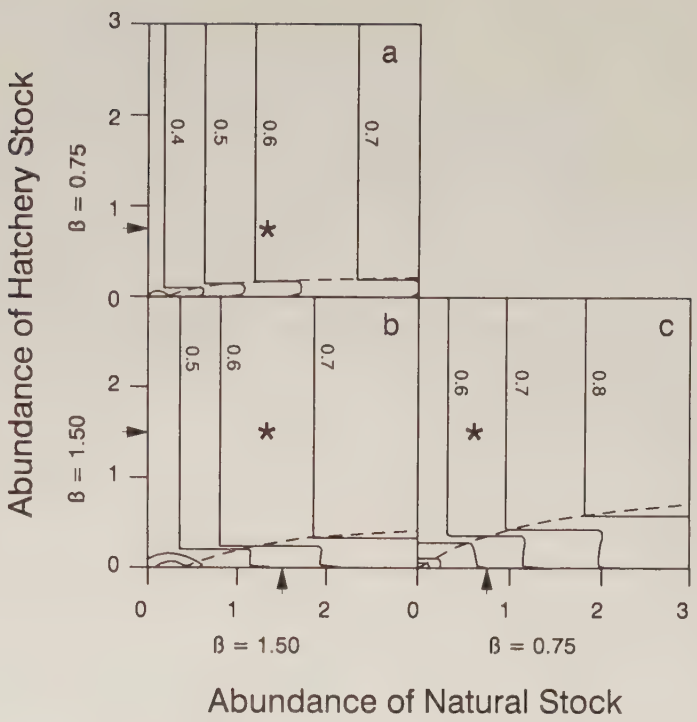

FIG. 7. Optimal risk-averse harvest rate contours for stocks with different production functions. The natural stock is governed by a Ricker SRR with moderate productivity ($\alpha = 1.5$), and the hatchery stock is governed by a high-productivity ($\alpha = 2.5$) rectilinear SRR. Individual stock MSY equilibria are indicated by arrows on the figure axes, and the joint MSY equilibria are indicated by asterisks. Isoharvest proportions occur along the broken lines.

(2) Over a fairly broad range of stock productivities, the stock dependence of both risk-neutral and risk-averse optimal harvest rates decreases as stock productivity increases.

(3) In hatchery-enhanced fisheries, jointly optimal harvest rates are more heavily influenced by the weaker stock over a fairly broad range of stock abundance combinations. The higher productivity of hatchery stocks ensures that the natural stock will nearly always be weaker than the hatchery stock.

While it is unlikely that stock forecasting and harvest regulation in mixed-stock fisheries will ever have the accuracy necessary to attempt to regulate harvest based on the relative abundance of individual stocks on a season by season basis, these axioms do have applicability to long-term management strategies. While short-term forecasting may be unreliable, it is widely accepted that a number of natural salmon stocks in California, Oregon, and Washington are chronically depressed (Nehlson et al. 1991). Overharvest has been identified as a factor contributing to the decline or hampering the recovery of most of these stocks. Preservation of genetic diversity and variability are often invoked as reasons for restoring depressed natural stocks, but the results presented here indicate that by criteria based on yield alone, these stocks should be restored. Harvest rates that optimize the expected yield from mixed-stock fisheries for hatchery and natural fish should routinely produce healthy runs of natural spawners and substantial surpluses of hatchery spawners. This implies that yields from fisheries that cannot discriminate between natural and hatchery stocks could be increased by reducing the harvest rates to rebuild depressed natural stocks.

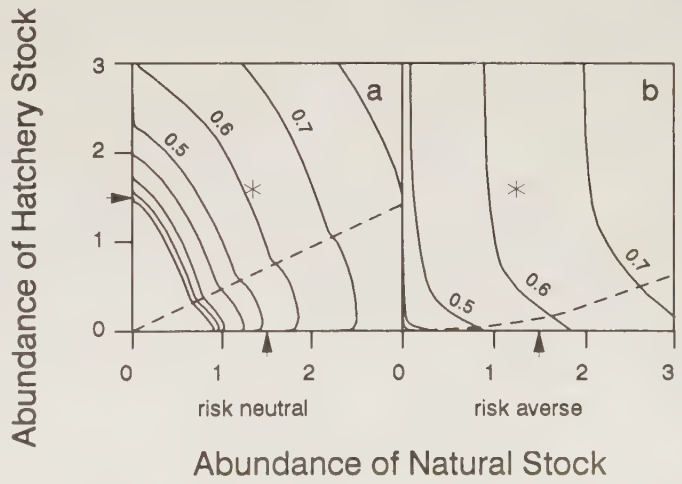

FIG. 8. Optimal harvest rate contours for a mixture of hatchery and natural stocks with hatchery stocks governed by a Beverton–Holt SRR. (a) Risk-neutral utility function, analogous to Fig. 6b; (b) risk-averse utility function, analogous to Fig. 7b.

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Histopathology of Rainbow Trout (*Oncorhynchus mykiss*) after Long-Term Exposure to Biologically Treated Bleached Kraft Mill Effluent in Experimental Stream Channels

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Outdoor experimental streams were used to determine the effects of effluent on histopathology of rainbow trout (*Oncorhynchus mykiss*). Studies were conducted for 10–11 mo using biologically treated bleached kraft mill effluent at concentrations ranging from 0.5 to 2.0 mg·L^{-1} of effluent BOD_5 (1.3–5.1% v/v) and for 42 mo at 0.5 BOD_5 addition (1.5% v/v). Twenty different tissues from randomly selected fish were examined at the end of each exposure period. Lesions or tissue changes observed in fish from both control and effluent-treated streams were of primary or secondary parasite-induced etiology, a condition typical of natural streams. There was an absence of neoplasia over the range of concentrations. Hematocrit, leucocrit, and liver somatic index remained normal throughout the course of effluent exposure. These data corroborate the lack of effluent effects as determined from measurements of trout growth, survival, production, and reproduction.

Des canaux expérimentaux externes ont été utilisés pour l'étude des effets histopathologiques d'effluents chez la truite arc-en-ciel (*Oncorhynchus mykiss*). Les essais ont porté sur des effluents d'usine de pâte kraft ayant subi un traitement biologique: de 10 à 11 mo à des concentrations variant de 0,5 à 2,0 mg·L^{-1} de DBO_5 (1,3–5,1 % v/v) et 42 mo à une DBO_5 de 0,5 (1,5 % v/v). Vingt tissus de types différents provenant de poissons prélevés au hasard ont fait l'objet d'un examen à la fin de chaque période d'exposition. Les lésions ou modifications histologiques observées, tant chez les témoins que chez les poissons exposés, correspondaient à une étiologie primaire ou secondaire s'expliquant par la présence de parasites, un état typique dans les cours d'eau naturels. Aucune néoplasie n'a été notée pour toute la gamme des concentrations d'essai. Les valeurs de l'hématocrite, de la leucocrite et de l'indice hépatosomatique sont demeurées normales tout au long de l'exposition aux effluents. Ces données corroborent l'absence d'effets dus aux effluents notée à partir de mesures de la croissance, de la survie, de la production et de la reproduction des truites.

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A variety of methods have been used to assess the potential adverse effects of pulp and paper mill effluents on fish health. One area of investigation has involved using histological, somatic, or blood cell parameters as indicators of environmental or physiological stress. A variety of approaches have been used over the years in studies with bleached kraft mill effluents (BKME). Although some studies have been carried out with biologically treated effluents, the majority reflect responses to effluents without secondary treatment. Additionally, few attempts have been made to identify whether or not these parameters correlate with changes in growth, survival, or production of fish. In this paper, results from a series of 10- to 42-mo exposures to BKME are presented, including evaluations of histopathology and production for fish in outdoor experimental streams.

The literature reports a variety of responses for fish exposed to BKME. Fish blood sugar, red blood cell, and white blood cell responses were reported by McLeay and Howard (1977) for juvenile coho salmon (*Oncorhynchus kisutch* exposed to untreated BKME. A follow-up study indicated that biological treatment of BKME was effective in reducing or eliminating effects based on the blood sugar analysis (McLeay 1977a). McLeay (1977b) found that rainbow trout (*Oncorhynchus mykiss*, previously *Salmo gairdneri*) leucocrit responses were ameliorated after biological treatment of BKME. Effluent treatment was also shown to significantly decrease plasma lactic acid, plasma glucose, and liver glycogen responses of *O. kisutch* (McLeay and Brown 1979). Fish growth was not significantly affected, although exposed fish had higher mean weights than controls.

Untreated BKME was used by Couillard et al. (1988) in tests with *O. mykiss*. Sublethal effluent test concentrations indicated no significant tissue changes attributable to effluent exposure. Effluent-exposed fish had higher prevalence of fin rot and gill lesions, which was thought to indicate increased environmental stress and/or an indication of increased susceptibility to bacterial infection.

Burton et al. (1983) exposed striped bass (*Morone saxatilis*) eggs and prolarvae to treated BKME at concentrations up to 20% v/v. A series of three egg hatching studies failed to indicate any effect of effluent on either mortality or hatch timing. Prolarvae from effluent-exposed eggs had no obvious morphological abnormalities.

Andersson et al. (1987) exposed fourhorn sculpin (*Myoxocephalus quadricornis*) to effluent in aquaria and measured the effects on muscle glycogen and liver somatic index. Results varied according to wood species, with fish exposed to untreated effluents from pine pulping showing significant increases in muscle glycogen and liver size. Hardig et al. (1988) reported that effluent reduced blood hematocrit, hemoglobin, and red cell count but did not alter white cell count.

Lehtinen and Oikari (1980) conducted studies on perch (*Perca fluviatilis*) exposed to untreated BKME. Various tissue anomalies were indicated, including increased cytoplasmic granularity, apically swollen secondary gill lamellae, and parasitic cysts in the secondary gill lamellae. Lehtinen et al. (1984) made similar observations with European flounder (*Platichtys flesus*) exposed to untreated BKME in artificial ecosystems. Fish exposed to effluent exhibited gill and liver structural differences when compared with reference fish from the effluent receiving water. Both receiving-water reference fish and microcosm-exposed fish exhibited similar trichodinid ciliate gill infestations.

To date, only a few studies with pulp and paper mill effluents have included both growth rate measurements and assessments of blood parameter or tissue changes. From the literature, it is unclear whether tissue abnormalities are reliable indicators of fish health as might be measured by more conventional fish health measurements such as growth, survival, or production.

The identified need to examine the relationship between conventional fish health measurements and histopathological changes prompted the National Council for Air and Stream Improvement (NCASI) to initiate a series of long-term studies in large outdoor experimental stream channels. The experimental streams were designed to function as natural ecosystems, allowing for the assessment of effluent effects on fish production directly as well as indirectly through the supporting food web. Studies were initiated at both a southern and northern location with biologically treated BKME to address concerns about both warmwater and cold-water fish species (NCASI 1983, 1989).

The cold-water stream studies are described here and were conducted on rainbow trout from 1980 to 1988. The experimental streams were exposed to a series of concentrations of biologically treated BKME for 10–11 mo at each concentration followed by an extended 42-mo study. The initial studies allowed for long-term measurements of fish production (growth and survival) and histopathology whereas the final study allowed additional time for in-stream spawning and completion of the life cycle.

This article focuses on fish histopathology and blood parameters with a brief synopsis provided on growth, survival, and reproduction. Supporting information relative to effluent exposure conditions and effluent chemical characterization is also provided. Information relative to periphyton and macroinvertebrate studies carried out in the streams and a more detailed description of the experimental stream facility and operating procedures of stream site have been reported elsewhere (Hall et al. 1991).

Materials and Methods

Experimental Stream Design

Four experimental streams, each 110 m in length, were constructed adjacent to the Clearwater River at the Potlatch Corporation in Lewiston, Idaho. Each stream consisted of an alternate pattern of 11 pools (1 m deep × 3 m wide × 4 m long) and 10 riffles (1.2 m wide × 6 m long). Water from the Clearwater River was pumped to the streamsite and regulated so that each stream received a mean water flow ranging from 859 to 1393 $L \cdot min^{-1}$ over the series of studies. The streams were nutritionally/biologically self-supporting, with 9 mo allowed prior to the initiation of studies for the streams to become colonized with food web organisms.

Effluent

Effluent was obtained from the outlet pump manifold of the aeration stabilization basin of the Potlatch Corporation and delivered to the streamsite through approximately 1300 m of 10-cm-diameter PVC pipe. The mill produces bleached kraft pulp from a variety of softwood species, primarily Douglas fir (*Pseudotsuga menziesii*), grand fir (*Abies grandis*), western red cedar (*Thuja plicata*), and white and ponderosa pine (*Pinus monticola* and *P. ponderosa*). The pulp is converted to tissue and paper on-site. A chlorine/alkaline extraction/hypochlorite/hypochlorite/chlorine dioxide (CEHHD) bleaching sequence is used for pulp from wood chips and a CEHD sequence for pulp from sawdust. Throughout the studies, water use averaged from 113 to 117 $m^3 \cdot t$ finished $product^{-1}$.

At the streamsite, effluent was distributed to two of the four streams. The same two streams (Streams 2 and 4) received effluent during each study period with the two remaining streams serving as controls (Streams 1 and 3).

Effluent was added to the two treatment streams based on the desired addition of BOD_5 for each study (Table 1). Daily BOD_5 values were obtained from the mill and used to determine the percent (v/v) addition of effluent to river water required to achieve the desired BOD_5 addition level.

Effluent additions to the experimental streams began at 0.5 $mg \cdot L^{-1}$ of BOD_5 and increased in yearly 0.5-$mg \cdot L^{-1}$ increments to 2.0 $mg \cdot L^{-1}$, the highest concentration tested. The final 42-mo study was conducted at the 0.5 $mg \cdot L^{-1}$ BOD_5 addition level. On a v/v addition basis, effluent to the treatment streams ranged from 1.3% for the initial study to 5.1% at the highest tested concentration (Table 1). Mean whole-effluent BOD_5 ranged from 35 to 44 $mg \cdot L^{-1}$ over the series of studies.

Two 2-d composite effluent samples were collected each week during the study period. Chlorophenol, resin, and fatty acid analyses were based on GC/MS procedures described by NCASI (1986a, 1986b). Additional water quality measurements relative to the interpretation of food web productivity are reported elsewhere (Hall et al. 1991).

The calculated in-stream concentrations for fatty acids ranged from 0.8 to 4.9 $\mu g \cdot L^{-1}$ over the progession of studies, with a

TABLE 1. Exposure periods and instream concentrations for effluent addition studies. Values are means, based on % effluent addition and measurements made on undiluted effluent. FA = fatty acid; RA = resin acid.

	Additions to treatment streams							
					Chlorinated			
Time (mo)	% v/v	BOD_5 ($mg \cdot L^{-1}$)	FA ($\mu g \cdot L^{-1}$)	RA ($\mu g \cdot L^{-1}$)	RA ($\mu g \cdot L^{-1}$)	Phenol ($\mu g \cdot L^{-1}$)	Guaiac. ($\mu g \cdot L^{-1}$)	Catech. ($\mu g \cdot L^{-1}$)
10	1.3	0.5	0.4	1.8	0.5	0.5	0.7	2.9
10	2.2	1.0	3.0	2.5	1.1	0.8	2.0	5.3
11	4.5	1.5	2.0	5.5	1.7	1.9	2.9	9.8
11	5.1	2.0	4.9	10.0	2.5	2.2	5.4	11.0
42	1.5	0.5	0.8	2.7	0.5	0.5	0.8	1.2

corresponding resin acid range of 1.8–10 $\mu g \cdot L^{-1}$ (Table 1). Mean chlorinated resin acid concentration ranged from 0.5 to 2.5 $\mu g \cdot L^{-1}$. Combined concentrations for the three forms of chlorinated phenolic compounds ranged from 3.7 to 21.1 $\mu g \cdot L^{-1}$. The chlorinated catechols made up the largest contribution to the chlorinated phenolics.

Stocking, Sampling, and Production Calculations

At the beginning of each study, streams were stocked with juvenile rainbow trout at a density of 3.7 $g \cdot m^{-2}$. Depending on the mean weight of fish, which ranged from 1.1 to 2.8 g for the five study periods, the number of fish stocked to achieve the specified density ranged from 234 to 600.

For growth determinations, fish were sampled monthly using a 3-mm-mesh seine and anesthetized with 25 mg MS222·L^{-1}. Fish were individually weighed to the nearest 0.01 g. Production and growth data were treated according to Chapman (1978).

Histopathology

At the termination of the initial four studies, 20 randomly selected fish from each stream were sacrificed for histopathological examination by the Environmental Pathology Laboratory, Inc., Carolina, RI. Due to greater fish losses during the 42-mo study, fewer fish were available for examination.

The histological assessment was based on 20 or more tissues from each fish. After anesthetization the coelom was opened and on fish larger than 10 cm long the brain case exposed, prior to immersion in Bouin's fixative at a volume 10 times or more the volume of the fish. After fixation for 72 h, fish were rinsed in cold water and sealed in plastic bags containing cotton wadding soaked with 10% neutral buffered formalin. Fixed fish were kept cool and in the dark until shipped to the pathologist. Fish fixation procedures were modified for the 4.5, 5.1, and 1.5% v/v effluent exposures by replacing Bouin's fixative with 10% neutral buffered formalin. Otherwise, procedures remained unchanged.

Fish were examined for gross lesions, dissected, and the tissues trimmed. Groups of tissues were placed with identifying numbers into processing cassettes, processed by standard methods, and embedded in paraffin. The embedded tissues were sectioned at 6 μm, placed on glass slides, and stained with hematoxylin and eosin. Blocks were then sealed with paraffin and stored for future reference. Tissues processed and examined with the light microscope were kidney, stomach, intestine, liver, pancreas, spleen, heart, gill, skin, pharynx, nares, muscle, bone, brain, eye, lateral line, thyroid, adrenal, head kidney, and gonad.

Blood Parameters

Blood leucocrit and hematocrit were determined during the final fish sample of each study. Ten fish were randomly selected from each stream with the exception of the 1.5% v/v exposure when all surviving fish were sampled. Following anesthetization and weighing, these fish were sacrificed with a blow to the head and the caudal peduncle then severed. Blood was collected immediately in microhematocrit tubes which were sealed with clay and centrifuged for 5 min at 11 500 rpm. The leucocrit and hematocrit were read and calculated according to McLeay (1977b).

Liver Somatic Index

A liver somatic index was calculated for fish at the final sample of the 1.5% v/v effluent exposure. Following anesthetization and weight measurement, livers were excised, blotted lightly with paper toweling, and weighed to the nearest 0.01 g. The somatic index was expressed as liver weight as a percentage of whole body weight.

Statistical Methods

The numbers of fish exhibiting a specific type of lesion from the two control streams were totalled and compared with the numbers of fish exhibiting the same type of lesion when exposed in the two effluent-treated streams. A 2×2 chi-square test ($p \leq 0.05$) was used to determine if any correlation existed between the presence of an abnormal tissue and the presence of effluent.

The percentage of the total blood volume occupied by the leucocrit and hematocrit of fish from control streams was compared with the leucocrit and hematocrit of fish from treatment streams using a t-test ($p \leq 0.05$). Similar statistical procedures were used in the interpretation of the liver somatic index.

Results

Histopathology

The incidence of the most common individual lesions for each of the exposures is provided in Table 2 with a summary of combined lesions provided in Fig. 1.

1.3% v/v effluent exposure

Fish were primarily affected by lesions of parasitic etiology. The number of lesions in fish from combined control streams

TABLE 2. Number of lesions or tissue changes in rainbow trout exposed to biologically treated BKME in experimental streams. Exposure conditions are expressed as % v/v effluent and lesion numbers as combined lesions for two replicate control and treatment streams (except for Study 5 when a single control and treatment stream was used due to lower fish numbers). Only major lesions or lesions considered to be of special interest are included.

	1.3% v/v		2.2% v/v		4.5% v/v		5.1% v/v		1.5% v/v	
Condition	Cont.	Treat.	Cont.	Treat.	Cont.	Treat.	Cont.	Treat.	Cont.	Treat.
Eye sanguinicoliasis	0	0	0	0	4	4	0	0	0	0
Gill epithyliocystosis	15[a]	4	3	0	4	0	2	0	0	0
Gill sanguinicoliasis	33	31	21	22	37	29	26	27	0	0
Gill trichodiniasis	0	0	0	0	0	0	5	7	0	0
Heart chronic myocarditis	0	0	0	2	12	13	0	0	0	0
Heart sanguinicoliasis	0	0	0	0	0	0	8	12	0	0
Intestine helminthiasis	21	24	4	9	0	0	2	4	1	2
Kidney tubular necrosis	0	0	0	0	26	18	2	5	0	0
Kidney hyperplasia	0	0	0	0	0	1	17	15	0	0
Kidney/adrenal hyperplasia	0	0	0	0	0	0	10	14	0	1
Liver glycogen depletion	8	12	11	11	0	0	0	0	0	0
Liver focal hepatitis	1	2	0	0	14	11	19	26	0	0
Liver hepatitis granulomata	0	0	4	2	0	1	0	0	0	0
Liver chronic hepatitis	0	0	10	8	0	0	0	0	0	0
Nares trichodiniasis	9	9	0	1	1	0	2	3	0	0
Nares chronic rhinitis	17	19	20	25	0	0	0	0	1	2
Nares vacuolization	0	0	6	14	0	0	0	0	0	0
Pancreas granulomata	0	0	1	0	0	0	0	0	0	0
Pharynx granulomata	0	0	0	1	0	0	0	0	0	0
Spleen hyperplasia	0	0	0	0	0	0	11	13	0	1

[a]Significant difference (chi-square, $p \leq 0.05$).

totaled 109 as compared with 104 lesions in treatment stream fish. Parasite-related lesions totalled 82 for combined control streams and 68 for treatment streams. The predominant parasitic lesion occurred in the gills, resulting from the blood parasite *Sanguinicola* sp. The incidence of gill lesions from this parasite ranged from 15 to 18 (75–90%) for the four streams, with the distribution indicating no relationship to effluent exposure.

Other parasitic or possibly secondary parasitic lesions or tissue changes included gill sanguinicoliasis, intestinal helminthiasis, and nares chronic rhinitis and trichodiniasis. The only significant difference in lesion distribution was for gill epitheliocystosis which indicated a higher incidence in control stream fish.

With the exception of lesions from *Sanguinicola* sp., all lesions were minimal in terms of severity and incidence and none suggested a pattern related to effluent treatment. The only observed tissue change of possible interest relative to effluent exposure was liver glycogen depletion. Glycogen depletion occurred in from zero to eight fish from the two control streams and from five to seven fish for treatments.

2.2% v/v effluent exposure

A total of 188 lesions were recorded, with 82 and 106 lesions observed in combined control and treatment stream fish, respectively. The decline in lesion numbers was primarily due to a large reduction in lesions of parasitic origin. The incidence of lesions induced by *Sanguinicola* sp. declined to 21 in combined control stream fish and 22 in combined treatment stream fish, a substantial reduction from 1.3% v/v effluent exposure. The severity of sanguinicoliasis was also markedly reduced, with relatively few fish considered to be more than minimally affected.

There were again a number of other parasite-related lesions including intestinal helminthiasis, nares trichodiniasis, chronic rhinitis, and nares vacuolization. There was an absence of significant differences in the incidence of lesions between control and treatment stream fish for either individual or combined lesions.

There was again an indication of a minor occurrence of liver glycogen depletion. During the 2.2% v/v effluent exposure the distribution and extent of glycogen depletion was uniform for fish across the four streams, supporting the conclusion that a relationship between glycogen depletion and effluent exposure was lacking.

4.5% v/v effluent addition

The total number of lesions was 109 in control stream fish and 84 in fish from treatment streams. A lack of significant differences was indicated for total lesions as well as individual lesions between control and treatment stream fish.

The frequency of parasitically induced lesions increased, with 77% of all lesions being associated with sanguinicoliasis or other parasitic origins. The level of parasitic lesions was comparable with that of the 1.3% v/v effluent exposure (70%) and well above that at 2.2% v/v effluent (41%).

Sanguinicoliasis accounted for 81% of all parasitic lesions, totaling 69 in control fish and 52 in treatments. The dominant impact site for sanguinicoliasis in both control and treatment fish was the gill, followed by the heart. The majority of these lesions were considered by the pathologists to be of minimal to moderate severity but not severe enough to compromise respiratory or organ function. The only other common lesions found were chronic focal hepatitis and chronic myocarditis, both probably related to *Sanguinicola* sp. and both occurring at relatively uniform levels in control and treatment stream fish.

5.1% v/v effluent addition

Lesions totaled 112 in combined control and 128 in combined treatment stream fish. There was again an absence of

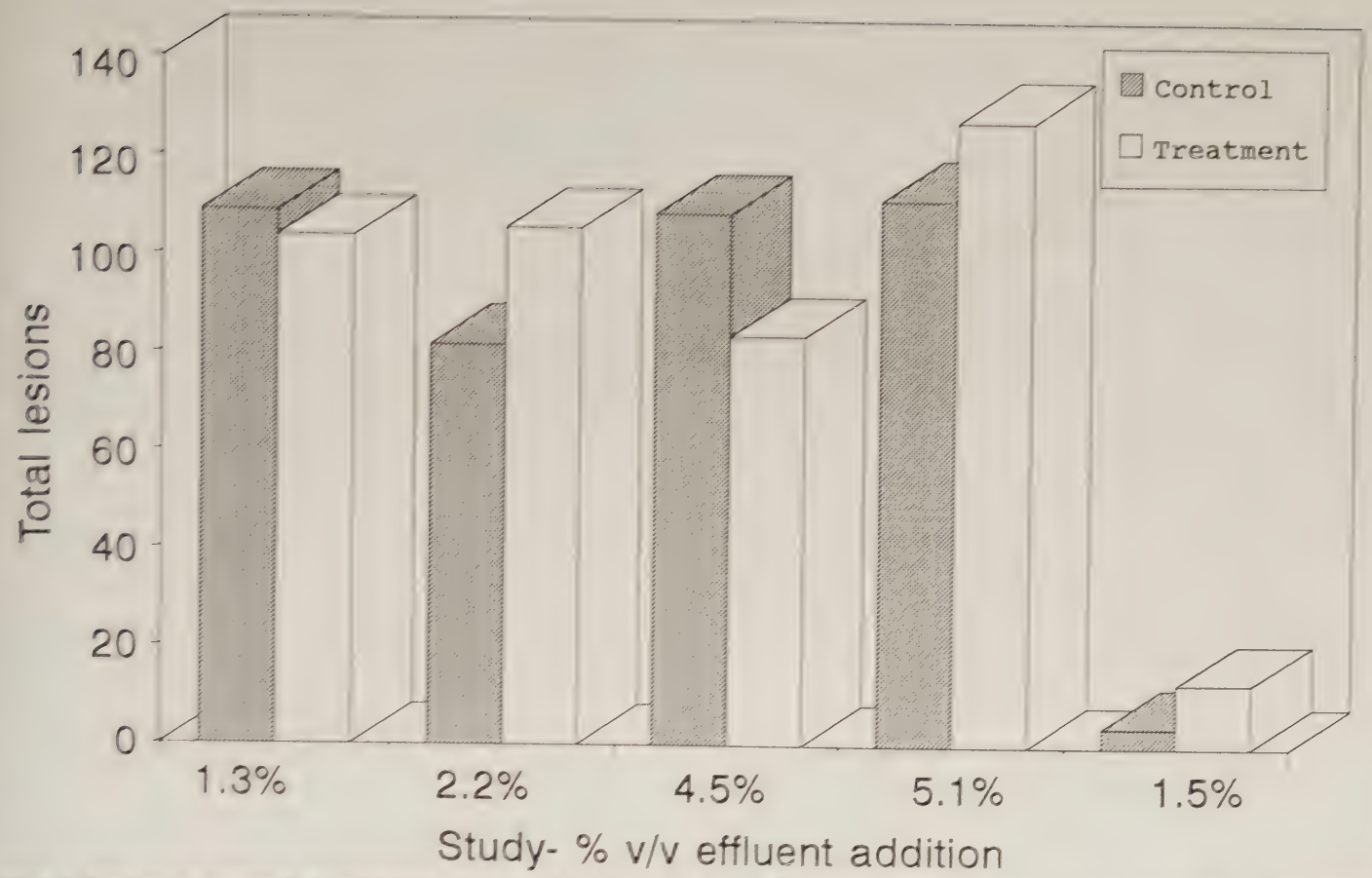

FIG. 1. Total lesion numbers in rainbow trout from control and treatment streams. Exposure conditions are expressed as % v/v effluent. Numbers are combined lesions for fish from two control and effluent treatment streams.

TABLE 3. Mean blood hematocrit and leucocrit values for rainbow trout from experimental streams.

	Hematocrit		Leucocrit	
Effluent % v/v	Cont.	Treat.	Cont.	Treat.
1.3	31.8	31.0	1.0	1.2
2.2	39.2*	44.0	1.7	2.0
4.5	41.7*	38.6	2.1	2.0
5.1	38.2	38.1	1.6	1.5
1.5	40.3	39.8	4.3	4.7

*Significant difference ($p < 0.05$).

significant differences in the incidence of individual or combined lesions between fish from individual streams or combined control and treatment streams. None of the lesions or tissue changes occurred in a frequency distribution pattern that would suggest an effluent effect.

Parasitic lesions declined from 77% in the 4.5% v/v effluent exposure to 54% in the 5.1% v/v effluent exposure, with lesions related to *Sanguinicola* sp. remaining the most common. The primary site of sanguinicoliasis was the gill, followed by the heart. Focal chronic hepatitis was also present in both control and treatment stream fish and was presumed to be related to sanguinicoliasis.

1.5% v/v effluent addition

Fewer fish (six control and five treatment) were evaluated due to reduced survival over the longer 42-mo exposure period. The number of lesions or tissue changes observed was four in control fish and 13 in treated fish, a difference that was significant.

Parasitic lesions accounted for three of four lesions in control stream fish and seven of 13 lesions in treatments. Brain mysoxpiridosis and gill copepodiasis were first encountered during this exposure. Additionally, the presence of gill epithelial hyperplasia, found in three of five treatment stream fish, may have also been an expression of copepodiasis.

Although the total lesion burden in fish from the treatment stream was greater than that in the controls the minimal severity of these lesions points to the association being coincidental rather than a real effluent exposure effect. Consistent with the other studies, there was an absence of neoplasia.

Blood Parameters

Hematocrit values were generally within the normal range reported for rainbow trout (Table 3) (Wedemeyer and Yasutake 1977). Significant differences between control and treatment stream fish occurred during the 2.2 and 4.5% v/v effluent exposures, but effects relative to effluent exposure were inconclusive. In one case, effluent exposure produced a higher hematocrit value and in the other a lower hematocrit value than in the corresponding controls. There were no significant differences in leucocrit values between control and treatment fish during any of the studies.

Liver Somatic Index

The mean liver somatic index for control stream fish during the final 42-mo 1.5% v/v effluent exposure was 1.17 as compared with 0.96 for treatment stream fish, a difference that was

not significant (ANOVA). The liver somatic index values for individual control stream fish ranged from 1.0 to 1.3 as compared with values ranging from 0.7 to 1.2 for treatment stream fish.

Discussion

Over the course of five studies, a total of 417 lesions or tissue changes were observed in control stream fish as compared with 441 in effluent treatment streams. These are totals for fish continuously exposed to effluent for periods ranging from 10 to 42 mo at effluent concentrations ranging from 1.3 to 5.1% v/v. Total lesions or tissue changes for fish in both control and treatment streams averaged between 2.3 and 3.0·fish^{-1} during the initial four studies; there were 1.5 lesions·fish^{-1} in the final 42-mo study.

There were no significant differences in the distribution of individual lesions between control and treatment stream fish that would indicate an effect of effluent exposure. Lesions related to parasitism totalled 282 in the control as compared with 275 in treatment streams, representing 68 and 62% of total lesions, respectively. The most common parasitic lesion was related to *Sanguinicola* sp. and accounted for 38 and 33% of total lesions in control and treatment fish, respectively. There did not appear to be a relationship between the occurrence of lesions and exposure to effluent.

Granulomata of the pancreas, liver, and pharynx, very likely lesions of parasitic origin, occurred only at very low frequency (five and six in control and treatment, respectively). Liver focal hepatitis, myocarditis, and hyperplasia of various organs may also have been secondary expressions of parasitism; these did not appear to be related to effluent exposure. Neoplasms (tumors) were lacking in both control and treatment groups.

Blood hematocrit and leucocrit values were within normal ranges for trout, with no indication of deleterious effects from effluent exposure. Significant differences in the liver somatic index between control and treatment fish were also lacking.

The results of histological and blood examination correspond well with results of fish survival, growth, production, and reproduction experiments carried out during the same series of studies (Hall et al. 1991). Rainbow trout in the experimental streams generally did not differ significantly from trout in control streams in cumulative fish production, although they tended to be larger and fewer in number. The successful spawning of fish exposed continuously to 1.5% v/v biologically treated BKME for 42 mo offers further verification of the lack of deleterious effects of the effluent, as does the successful survival and growth of their offspring.

These results differ considerably from some reported in the literature. The differences in reported responses are most likely related to the degree of effluent treatment. Studies in which blood changes (Hardig et al. 1988), enlarged livers, tissue damage, and heavy parasite loads (Lehtinen and Oikari 1980) are reported for several Baltic Sea fish were conducted using untreated effluent. The observations of blood cell changes documented by McLeay and Howard (1977) were also made on fish exposed to untreated effluent. McLeay (1977a, 1977b) and McLeay and Brown (1979) documented the utility of secondary treatment in ameliorating the effects of effluent on blood cell characteristics and several tissue parameters with several salmonids. Burton et al. (1983) further established the nonharmful nature of effluent exposed to secondary treatment in early developmental studies with *M. saxatilis*.

The series of long-term studies NCASI (1983, 1989) with both warmwater and cold-water fish and their supporting food web further illustrate the value of secondary treatment in reducing the potential for harmful effects of effluent. The inclusion of data on histopathology and blood parameters during these studies provides additional assurance that long-term effects, not discernable through measurements of fish production, are not taking place.

Acknowledgements

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Evolution of Dissolved and Particulate Matter during the Ice-Covered Period in a Deep, High-Mountain Lake

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Catalan, J. 1992. Evolution of dissolved and particulate matter during the ice-covered period in a deep, high-mountain lake. Can. J. Fish. Aquat. Sci. 49: 945–955.

Changes in inorganic and organic matter beneath the ice in a deep oligotrophic lake are used to establish temporal and spatial scales of physical and biological processes involved in the dynamics of the system during low energy flow. Four phases were distinguished: (1) Ice-forming phase; light still penetrated the relatively thin snowpack, production was high, and a chlorophyll maximum developed close to the ice. (2) Transition towards a dark environment; light was reduced to very low levels by rapid snow accumulation, phytoplankton showed symptoms of shade adaptation, and nutrients and dissolved organic compounds changed markedly (e.g. soluble reactive phosphorus increased). (3) Central phase; for several months, loss processes (respiration, sedimentation) maintained constant rates, three zones of differing variability patterns were distinguished in the water column: an upper zone where biomass was higher and which was affected by exchange of substances with the cover during flooding processes, a middle zone with little change throughout the phase, and a deep layer, identified by catabolic activity, where diffusion of compounds from the sediment took place. (4) Thaw; sequential changes occurred near the surface owing to snowpack melting, chlorophyll reached the lowest winter values of the period in spite of light and nutrients, and nitrogen compounds changed significantly.

Les changements dans la matière organique et inorganique sous la glace dans un lac oligotrophe profond ont été utilisés dans l'établissement d'échelles temporelles et spatiales des processus physiques et biologiques participant à la dynamique du système en période de flux énergétique faible. Quatre phases ont été repérées: 1) La phase de formation de la glace; durant laquelle la lumière traversait encore la couche de neige relativement mince, la productivité était élevée et la concentration de chlorophylle était maximale près de la glace. 2) La phase de transition vers l'obscurité; durant laquelle l'intensité lumineuse a été réduite à un très bas niveau en raison de l'accumulation rapide de neige, le phytoplancton montrait des signes d'adaptation à cette noirceur et les quantités d'éléments nutritifs et de composés organiques dissous a varié de façon marquée (par exemple, le phosphore réactif soluble s'est accru). 3) La phase centrale; d'une durée de plusieurs mois, durant laquelle les taux de respiration et de sédimentation sont restés constants et on a pu distinguer dans la colonne d'eau trois couches bien caractérisées: la couche supérieure, où la biomasse était la plus élevée et qui était touchée par des échanges de substances avec la couverture durant les processus de débordement des eaux, la couche moyenne, qui a montré peu de changements durant toute la phase et la couche profonde, caractérisée par une activité catabolique, où il y a eu diffusion de composés contenus dans les sédiments. 4) La phase de dégel, durant laquelle on a observé près de la surface des changements séquentiels causés par la fonte du couvert neigeux, la chlorophylle était à ses plus basses concentrations de l'hiver malgré la présence de lumière et d'éléments nutritifs et les concentrations des composés azotés ont changé de façon importante.

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High-mountain lakes (like hot springs, salt lakes, and polar lakes) provide recognized opportunities for study because of their relative ecological simplicity. Most of them are covered by ice for part of the year, but little advantage has been taken of this opportunity for research, and basic information is still scarce. Formation of surface ice on a lake eliminates wind action and reduces heat exchange, so that vertical transport is reduced. Snow accumulation on the top of the ice sheet also reduces light penetration. The dynamics of the pelagic system depend on initial matter in the water column and on the system's capacity to reduce losses and enhance recycling. Although ice forms on many lakes above 40°N and on many high-mountain lakes below this latitude, limnology of the ice-covered period is not as well understood as that of the ice-free period (Wetzel 1983; Margalef 1983). The lack of information is more significant for deep oligotrophic lakes (Rodhe et al. 1966; Tilzer and Schwartz 1976); studies usually concern relatively shallow lakes (Kerekes 1974; Capblancq 1972), some of which show very particular features, like permanently covered Antarctic lakes (Goldman et al. 1967; Vincent 1981). This paper deals with the ecosystem dynamics of Lake Redó, Spain, during the period of ice cover. This high-mountain lake has geomorphological characteristics that allow the study of under-ice processes without significant littoral influences and without complexity added by advective or inertial movements which increase variability and complicate sampling design and the interpretation of results. The purpose of this investigation is to use changes of dissolved and particulate matter to determine spatial and temporal scales of physical and biological processes involved in organic matter decay and regeneration of nutrients under ice in an oligotrophic lake.

Study Site

Lake Redó (42°38′34″N, 0°46′13″E) is a cirque lake located in the Central Pyrenees (Spain) at 2240 m a.s.l. Morphology,

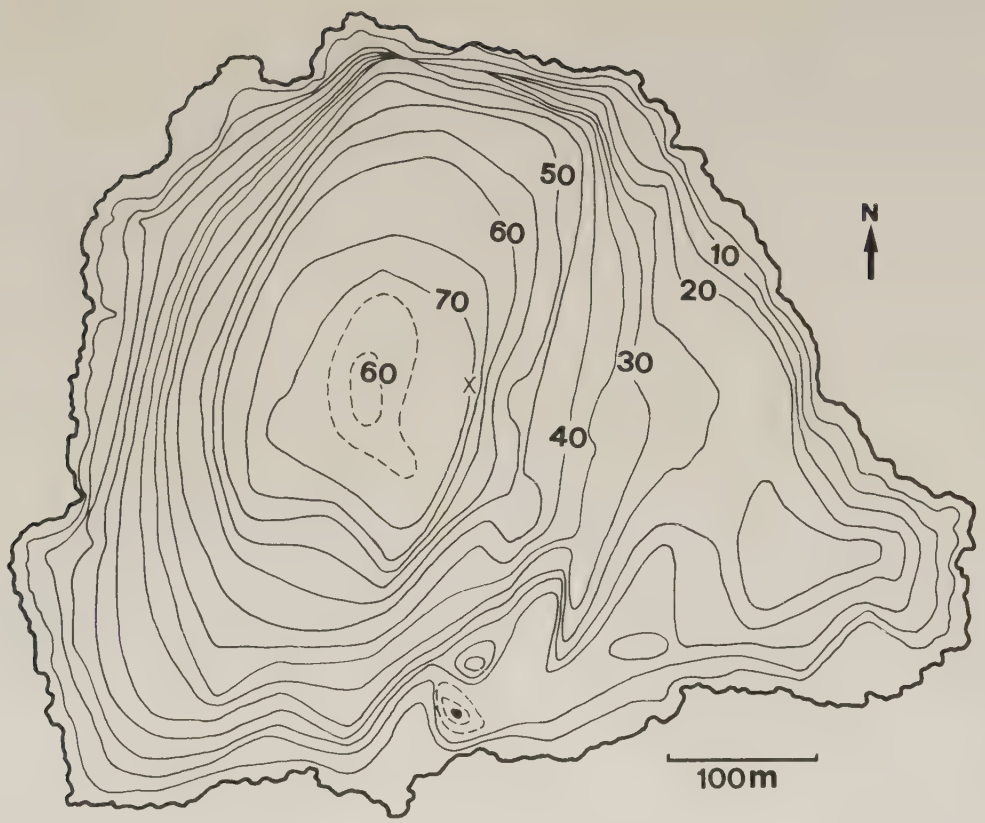

FIG. 1. Bathymetric map of Lake Redó, indicating the location of the sampling station (×).

characteristics of the watershed, annual thermal cycle, and mixing dynamics are described in Catalan (1988). The lake is deep (maximum depth 73 m; mean depth 32 m) in relation to its surface area (24 ha) (Fig. 1). The lake water has a conductivity of 10 $\mu S \cdot cm^{-1}$ and an alkalinity of 30 $\mu eq \cdot L^{-1}$ because of the resistance to weathering of the granodiorite rock. Mean epilimnetic total phosphorus is <0.15 $\mu mol \cdot L^{-1}$ (Catalan and Camarero 1991); thus, the lake is clearly oligotrophic (Vollenweider 1968). Sediment redistribution processes are dominated by the steep slope of the basin (Håkanson 1982; Hilton 1985); fine sediment concentrates below 25 m and especially below 40 m (Catalan 1988). Peaks of primary production occur when the mixed layer reaches these sediment-rich depths. The lake is dimictic and most of the production occurs during the fall overturn (Catalan and Camarero 1991).

The watershed is a small (131 ha excluding the lake) and steeply sloped with 90% of the drainage area below 2500 m a.s.l. The low ratio of the drainage area to the volume of the lake (7.75×10^6 m^3) results in an average residence time of the water in the lake of more than 4 yr (Catalan 1988). The lake receives water from multiple points, channelled through the fractures and irregularities of the catchment. There are no permanent streams in the basin; most of the inlets are active only during thaw and briefly after torrential rain in summer or fall. There is only one surface outlet. Soil is poorly developed in the whole watershed. A high percentage of the basin is bare rock or a lithosol where lichens and mosses take root. The rest is ranker soil with herbaceous vegetation.

Winter weather and the physical and chemical structure of the snow and ice cover for the period studied have been described in detail in Catalan (1989). Night air temperatures were below 0°C from November to mid-June. Maximum daily temperatures oscillated markedly; during January and March, they were always below 0°C, and in February, they reached positive values on a few days. The lake froze in mid-December and outflow began in late May; there was no flow during most of the period studied.

Methods

The sampling period was from the fall isothermy of 1984 to the spring isothermy of 1985. Sampling dates were spaced according to the physical changes in the winter cover. From overturn to the first major snowfalls, the lake was visited every 15 d, during winter every 20–30 d, depending on the weather conditions, and during thaw every 5–7 d. Samples were taken over the deepest part of the lake, using a Ruttner bottle with opaque body and dark subsample bottles to avoid light-flashing of the samples.

Conductivity was determined with a PT1-10 conductimeter and pH with an Orion Research model 231 pH meter. A low ionic strength filling solution was used in the pH electrode (Orion RX combination pH 91-65), and the recommendations of Neal and Thomas (1985) for pH measurements in low ionic strength waters were followed. Dissolved oxygen was determined by a modified Winkler method (Grasshoff et al. 1983). Alkalinity was determined with a Gran titration (Edmond 1970) following the algorithm of Catalan and Camarero (1987) in the calculations. Calcium, magnesium, and manganese were determined in acidified subsamples by induced coupling plasma spectrometry (Jobin Ivors V.H.R.). Sodium and potassium were determined by flame emission spectrometry (PYE Unicam SP1900) adding LiCl (final concentration 500 $mg \cdot L^{-1}$) to avoid interference. Ammonium nitrogen (NH_4^+) was determined

using the phenolhypochlorite method (Solórzano 1969), a phenol–nitroprusside–buffer reagent being added to subsamples in the field. Nitrate (NO_2^-), nitrate (NO_3^-), total dissolved nitrogen (TDN), soluble reactive phosphorus (SRP), total dissolved phosphorus (TDP), and particulate phosphorus (PP) were determined following Grasshoff et al. (1983). Some modifications were introduced in the analytical procedure of phosphorus fractions: (a) in SRP determinations, 250 mL of sample was concentrated to 13 mL using *n*-hexanol; (b) in TDP and PP determinations, after acid peroxidisulfate digestion of the sample, 50 mL was concentrated to 4 mL; (c) ethanol was found to be more suitable than isopropanol to clarify *n*-hexanol after concentration; (d) absorbance readings (Shimatzu 240 spectrophotometer) were allowed to stabilize for 15 min, and then repeated readings were carried out for 5 min and the mean value was used for determination. Furthermore, great care was taken to maintain material and reagents free of phosphorus, and blanks of all reagents employed were always determined. Standards were run with each sample series. Particulate carbon (PC) and particulate nitrogen (PN) were determined with a Carlo-Erba nitrogen analyzer 1500. Filtration was carried out onto previously ignited Whatman-GF/C glass-fiber filters and the first 250 mL was discarded before using filtered water in fraction analysis. Filter blanks of PP, PN, and PC were determined until the stabilization of the variance. Water for determinations not requiring fractionation was not filtered.

Chlorophyll (Chl) was determined using the spectrophotometric method of Jeffrey and Humphrey (1975). No acidifying corrections (Lorenzen 1967) were made; instead the A430/A410 ratio (Moss 1967) was used as a phaeopigment indicator. An HPLC chlorophyll determination was carried out on 20 May 1985, following a methanol method based on Falkowski and Sucher (1981) adapted to our equipment (Kontron). The relation between the A430/A410 and molar ratio between Chl *a* and total Chl *a* related pigments (tChl *a*) was calibrated with lake samples (A430/A410 = 0.62 + 0.483 Chl *a* / tChl *a*; $r^2 = 0.768$, $p < 0.001$). The A480/A665 ratio was used as an indicator of the relative abundance of carotenoids (Strickland and Parsons 1968).

Lakewide mass changes of certain compounds were calculated as follows:

$$C = 1/A \int_0^{z_{max}} A_z C_z dz$$

where C is the average quantity of some compound per unit of lake surface, C_z and A_z are the concentration of the compound and the area at depth z, and A is the lake surface area. Rates of change were calculated by linear regression of mass per unit of lake area versus time.

Results and Discussion

Pennak (1968) pointed out that total thickness of the winter cover of lakes is meaningless for understanding the dynamics in the water column and emphasized the significant role of the structure of the cover formed by several layers of different chemical and physical nature. The cover structure of Lake Redó for the period considered here has been described in detail by Catalan (1989). Four periods in the structure of the cover of ice and snow were distinguished (Fig. 2): (1) the lake surface froze and there was a sudden transition from a highly turbulent environment to a situation that excluded wind action on the surface but not light, as absorbtion by black ice was low; (2) snow accumulated on the ice and reduced light penetration to very low levels; (3) conditions of inappreciable convection and very low light levels persisted for several months, and (4) the thaw. Periods 1 and 2 were short in relation to periods 3 and 4 and were characterized by starting with a sudden change in the pelagic environment which took place at a shorter time scale than the lifetime of most planktonic microorganisms. I use these periods as a guideline to describe changes of dissolved and particulate matter in the water column.

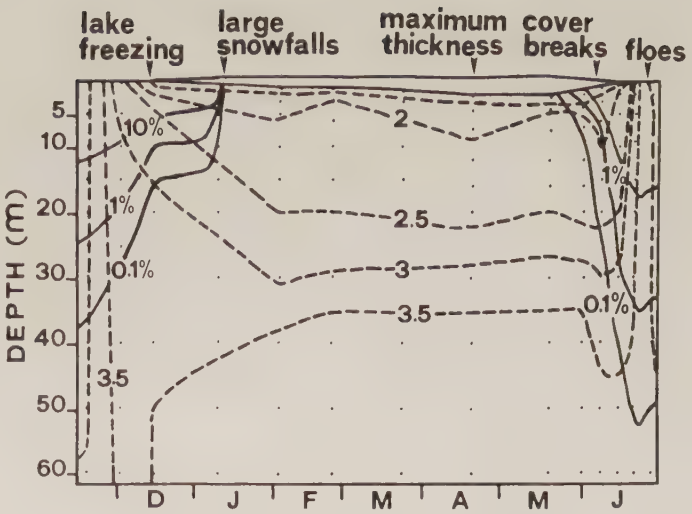

FIG. 2. Temperature (°C) (broken lines) and light (solid lines) isopleths for the ice-covered period in Lake Redó, 1984–85. Light is % PAR at depth with respect to water or cover surface. Cover thickness is scaled; its measured maximum was 2.5 m on 19 April. Main events in the cover dynamics are marked.

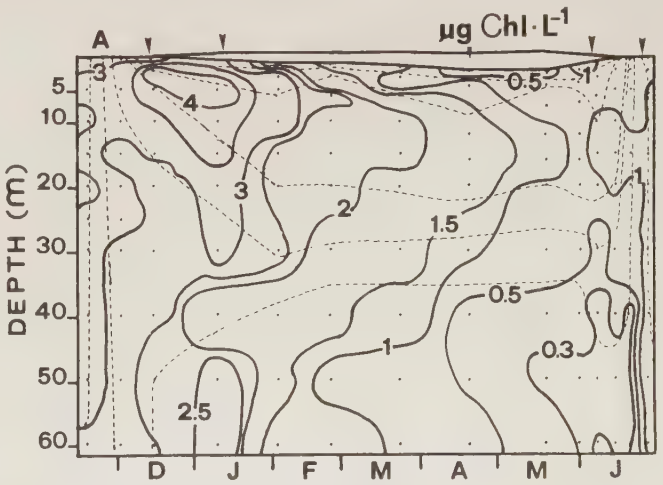

FIG. 3. Chl isopleths for the ice-covered period in Lake Redó, 1984–85. Cover events (arrowheads) and isotherms (broken lines) are shown as in Fig. 2.

(1) Ice Formation Period (from mid-December to mid-January)

When the lake was free of ice, mixing of the column took place several times per day (apparent coefficient of eddy diffusion, Kz = 10^{-1} $m^2 \cdot s^{-1}$) because of high wind speeds (up to 15 $m \cdot s^{-1}$) and strong convective mixing due to the intense loss of heat (>100 $W \cdot m^{-2}$) (Catalan 1988). When the lake

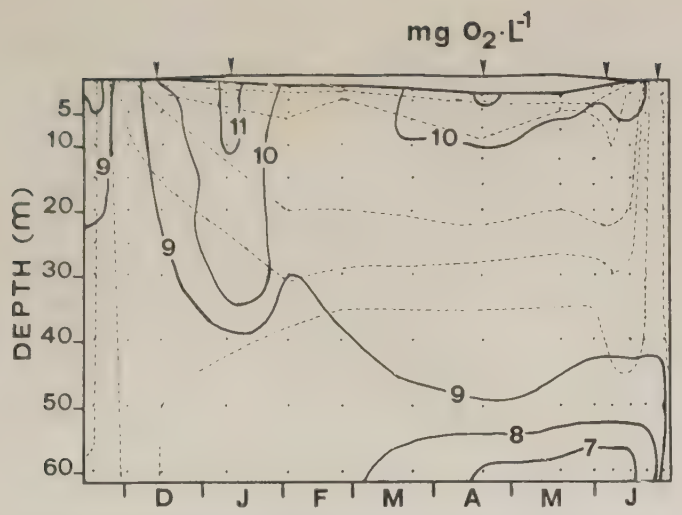

FIG. 4. O_2 concentration isopleths for the ice-covered period in Lake Redó, 1984–85. Cover events (arrowheads) and isotherms (broken lines) are shown as in Fig. 2.

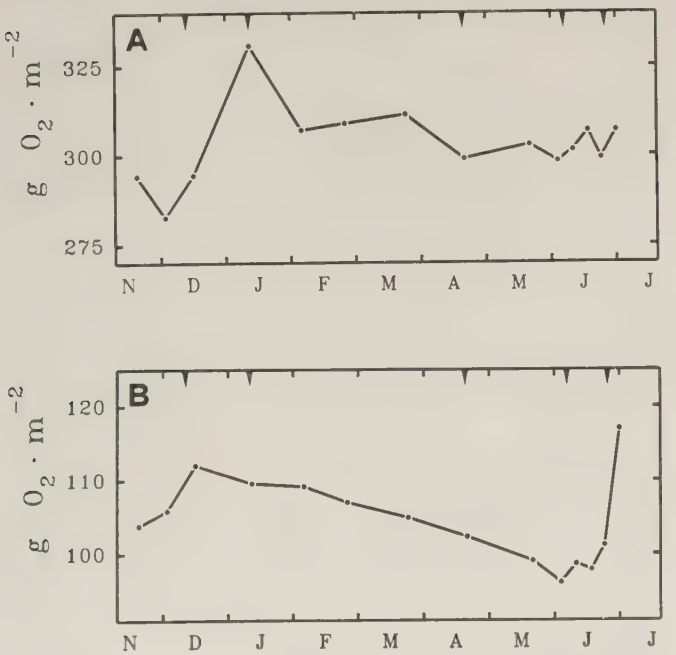

FIG. 5. (A) Mean lakewide O_2 concentrations and (B) mean O_2 concentrations below 50 m during the ice-covered period in Lake Redó, 1984–85. Cover events (arrowheads) are shown as in Fig. 2.

froze, on a cold calm night (14 December), there was a sudden decrease in water turbulence; Kz decreased by at least two orders of magnitude. Wind action was stopped and heat loss decreased to about 10 $W \cdot m^{-2}$ (Catalan 1989). The sediments, heated during summer, continued to lose heat, resulting in a downward convective movement as the water temperature was below 4°C. Radiation through the transparent ice may also have induced convective movement. In Fig. 2, heating from sediments is shown in the behaviour of the 3.5°C isotherm. From the time the lake froze, this isotherm gradually moved upward and stabilized at about 35 m at the end of February.

From the middle of August until the accumulation of snow on top of the ice in mid-January, light penetration was controlled by phytoplankton biomass (Catalan 1988). The formation of ice did not alter the relationship because black ice

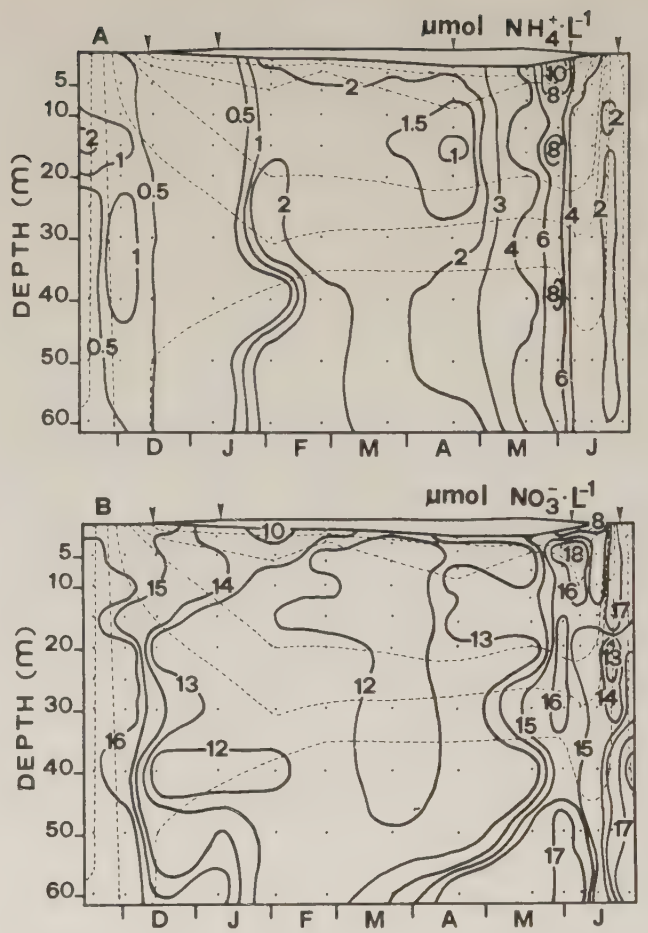

FIG. 6. NH_4^+ and NO_3^- isopleths for the ice-covered period in Lake Redó, 1984–85. Cover events (arrowheads) and isotherms (broken lines) are shown as in Fig. 2.

absorbed little radiation (extinction coefficient (0.004–0.015·cm^{-1}) (Catalan 1988). There was a phytoplankton bloom in the upper water column immediately after freezing and before the first heavy snows, with Chl reaching 4.7 $\mu g \cdot L^{-1}$ at 5 m depth (Fig. 3). One per cent of the surface incident radiation reached a depth of about 25 m during overturn and 10 m when the lake froze.

In addition to the increase in Chl, an O_2 increase (Fig. 4, 5) and a NO_3^- decrease (Fig. 6, 9) indicated high production during this initial period. The O_2 increase below the ice during the snow-free period (two sampling dates) was 1.4 g $O_2 \cdot m^{-2} \cdot d^{-1}$ (Fig. 5). O_2 freeze-out during black ice formation (35 cm of ice of density 917 $kg \cdot m^{-3}$) accounted for 0.1 g $O_2 \cdot m^{-2} \cdot d^{-1}$; thus, assuming no other source of O_2, net production in the water column in this period would have been 1.3 g $O_2 \cdot m^{-2} \cdot d^{-1}$. NO_3^- peaked during fall overturn and progressively decreased until the end of January (Fig. 6). The rate of NO_3^- decrease in the period of O_2 increase below ice was 1.1 $mmol \cdot m^{-2} \cdot d^{-1}$. According to the stoichiometry of Stumm and Morgan (1981), these changes in O_2 and NO_3^- indicated a production between 0.1 and 0.3 g $C \cdot m^{-2} \cdot d^{-1}$, which are common values during periods of production maximum in other oligotrophic Pyrenean lakes (Capblancq 1972) and Austrian Alpine lakes (Findenegg 1964; Tilzer 1972).

The seasonal trends of variation in PC and PN were similar throughout the period studied (Fig. 7). From maxima during the fall overturn, values decreased steadily until the end of the

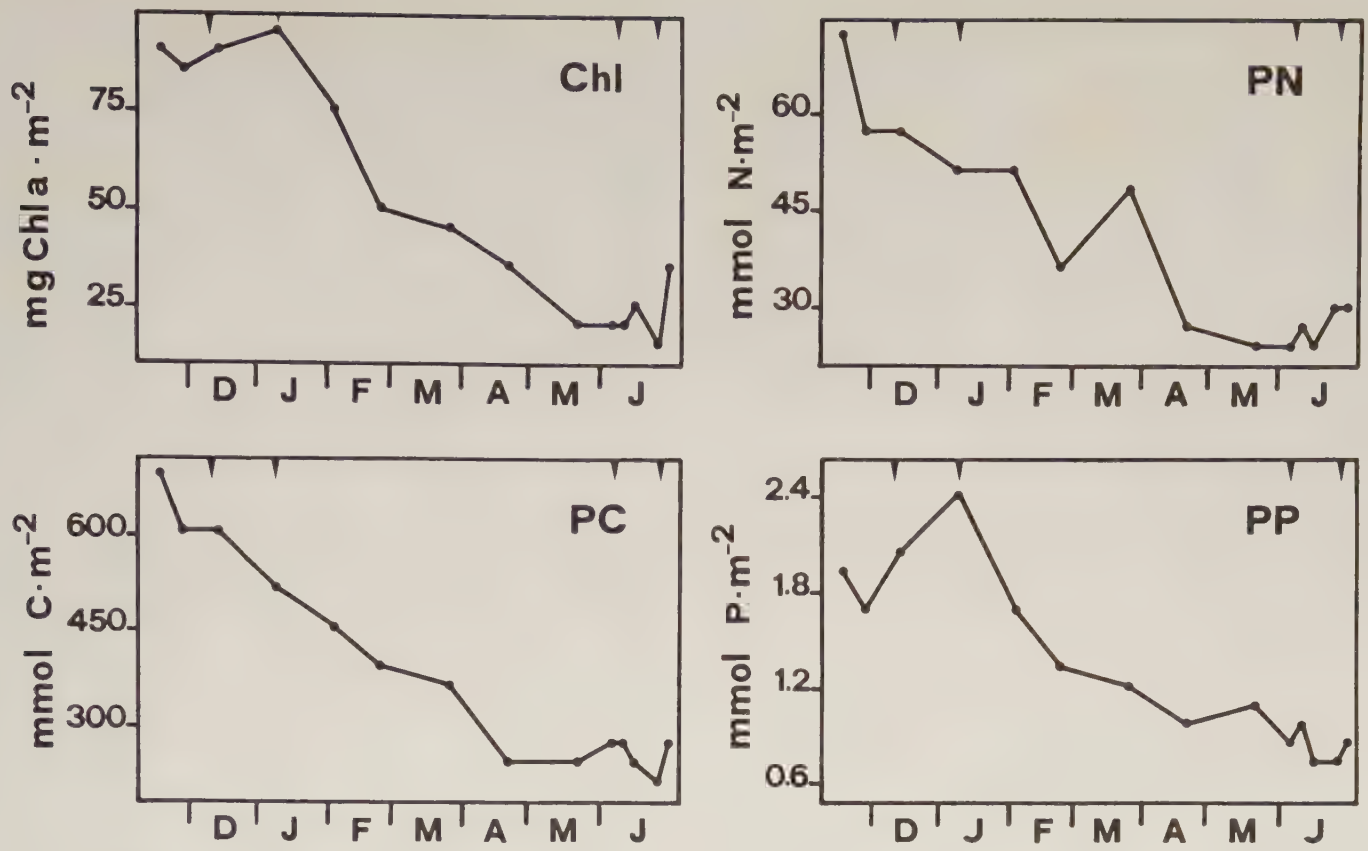

FIG. 7. Mean Chl, PN, PC, and PP per unit of lake area during the ice-covered period in Lake Redó, 1984–85. Main events (arrowheads) are shown as in Fig. 2.

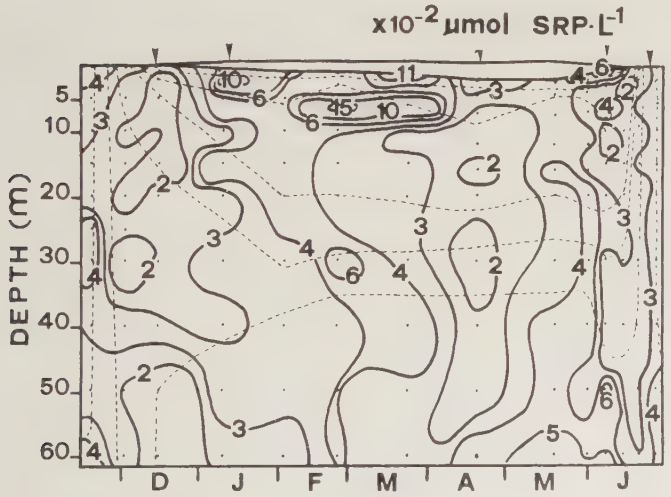

FIG. 8. SRP isopleths for the ice-covered period in Lake Redó, 1984–85. Cover events (arrowheads) and isotherms (broken lines) are shown as in Fig. 2.

thaw. In contrast, PP and Chl were highest beneath the ice when heavy snowfalls began (Fig. 7). Changes in PC:PP (from 350 to 260 by atoms) and PN:PP (from 35 to 25) can be explained either by the sinking of detrital material rich in PC and PN, which had been resuspended in the water column during overturn, or by a high growth rate of phytoplankton (Goldman 1980). Changes in O_2 and NO_3^-, increase of Chl, and the fact that SRP values were the lowest found during the period studied (Fig. 8) favour the latter hypothesis.

(2) Transition toward a Low-Light Environment

On 11 January 1985, substantial accumulation of snow began (30 cm new snow·d^{-1}). Snow has a higher light extinction coefficient (0.2–0.9·cm^{-1}) (Catalan 1988) than ice and reflects a higher percentage of incident light (70–90%) (Ragotzkie 1978). Light in the water column was reduced to levels lower than 0.1% of snow-surface irradiance in less than 3 d (Catalan 1988). An increase in the Chl *a*:(Chl *b* + Chl *c*) ratio from 20 to 30 by weight, especially in the shallowest layers, indicated a shade adaptation of the phytoplankton populations (Falkowski 1980; Osborne and Raven 1986). The low PC:Chl ratio (<60 by weight) between 5 and 30 m could be interpreted in the same way, as observed in other high-mountain lakes (Tilzer and Schwartz 1976).

As snow cover increased, SRP, which was between 0.01 and 0.03 µmol P·L^{-1} throughout the water column during fall overturn and early ice period, increased to values above 0.04 µmol P·L^{-1} and even higher than 0.1 µmol P·L^{-1} in the upper 10 m of the water column (Fig. 8). During the first month after freezing, high figures of soluble nonreactive phosphorus (SNRP = TDP − SRP) (≥0.25 µmol·L^{-1}) occurred between 5 and 10 m and between 25 and 30 m. At the beginning of February, similar high values occurred throughout the column, but when SRP increased in mid-February, there was a marked decline of SNRP to <0.15 µmol·L^{-1}.

After the lake froze, dissolved organic nitrogen (DON) increased to a maximum when heavy snowfalls began and then decreased markedly in a short time (Fig. 9). At this time, NO_2 peaked (Fig. 10) in the first few metres under ice where Chl was high (Fig. 3), and NH_4^+ increased from <0.5 to 2 µmol·L^{-1} in the whole column.

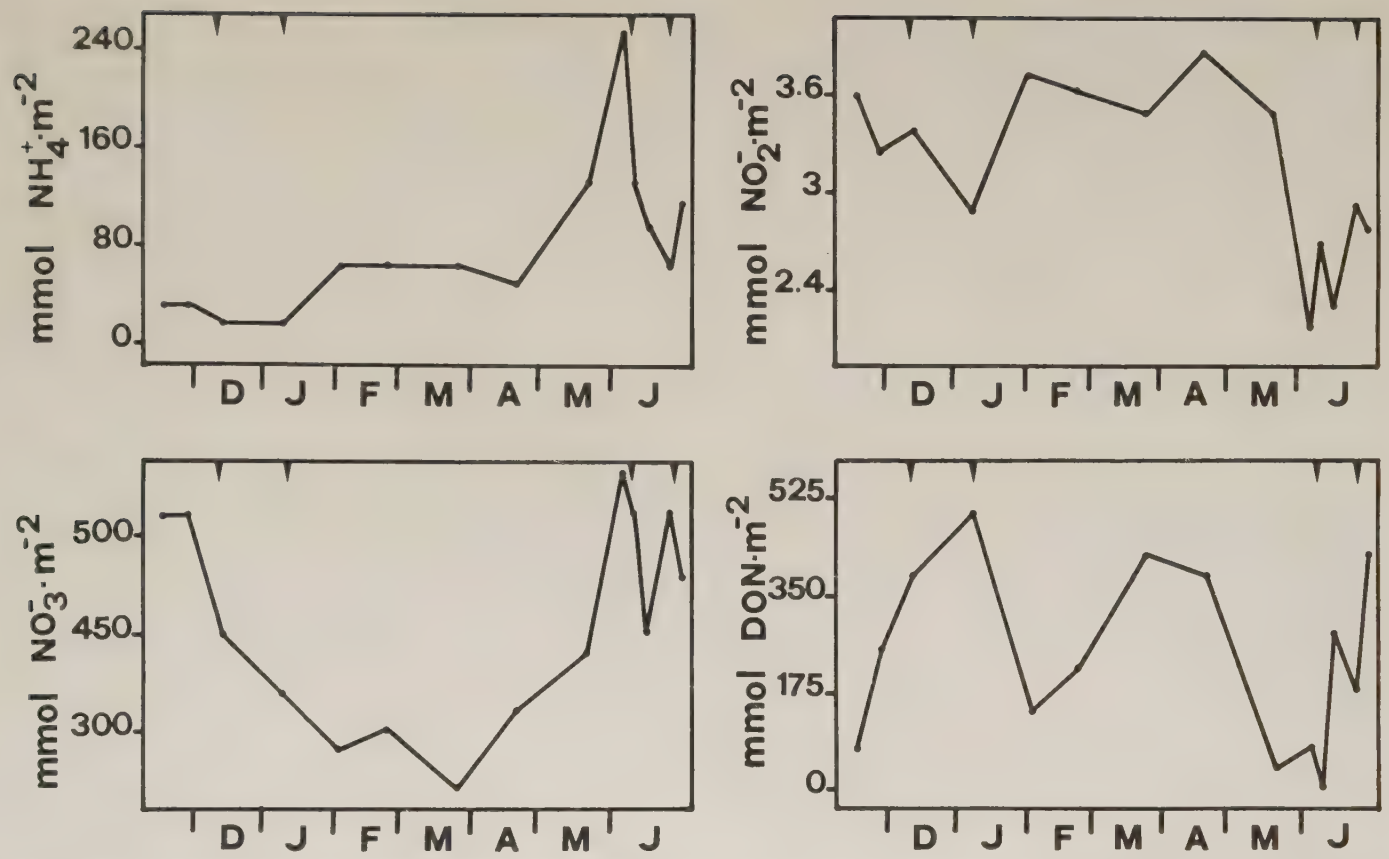

FIG. 9. Mean concentrations of nitrogen compounds per unit of lake area during the ice-covered period in Lake Redó, 1984–85. Main events (arrowheads) are shown as in Fig. 2.

(3) Central Phase of the Ice-Covered Period

From mid-January to mid-May, irradiance under the ice was <0.1% of that at the snowpack surface (Catalan 1988). The studies of Welch in Ontario and arctic lakes (Welch 1974; Welch et al. 1976; Welch and Bergmann 1985b) show that winter respiration rates are constant. In Lake Redó, apparent respiration rate from large snowfalls to thaw was 0.206 g $O_2 \cdot m^{-2} \cdot d^{-1}$ (Fig. 5), similar to that found in Ontario lakes (Welch et al. 1976). The determination of the rate is not precise ($r^2 = 0.61$, $p > 0.1$, $n = 8$), probably because changes in O_2 concentrations in the layers that contribute most to integration (layers at depths of less than 30 m) were minimal and these layers were also influenced by exchanges of water with the cover (see below and Catalan 1989). O_2 transport by water which flooded the cover from the lake (see below) would be about 0.09 g $O_2 \cdot m^{-2} \cdot d^{-1}$. Assuming no other O_2 exchange across the cover, respiration rate would be 0.197 g $O_2 \cdot m^{-2} \cdot d^{-1}$.

Changes in O_2 concentration below a depth of 30 m were more linear (Fig. 5B). When the isotherms were stable from March to the beginning of June, it can be assumed that below that depth, respiration was mainly due to biological processes because mixing time would be longer than sampling periodicity, although there could be some vertical mixing without changes in temperature (Welch and Bergmann 1985a). Mixing time (h/q^*) can be estimated from the expression $h/q^* = h^2/Kz \cdot h^{-1}$ where Kz is the coefficient of eddy diffusivity, q^* is the mixing velocity, and h is an appropriate length of mixing (Imboden et al. 1983). In our case, Kz could be assumed to be 10^{-6} or 10^{-5} $m^2 \cdot s^{-1}$, and the length of mixing could be the ratio between the volume below 30 m and the area at this depth ($h \approx 5$ m). These assumptions give a mixing time from 1 mo to 1 yr; therefore, changes in O_2 concentration in a given layer can be assumed to be caused by biological activity and not by physical transport. Since the organic matter content and temperature were very uniform in all the layers below 25 m (Fig. 11), respiration in the water column could be taken as constant. The O_2 consumption rate (milligrams O_2 per cubic metre per day) in a stratum was related to the ratio of the sediment surface area in the stratum (SS, square metres) to the volume of the stratum (SV, cubic metres):

$$\Delta O_2 = 85.1\ SS/SV + 0.0028$$

$$(r^2 = 0.93, p < 0.03, n = 5).$$

The rate of O_2 consumption (ΔO_2) was calculated by linear regression of O_2 concentration in the stratum against time.

In the above equation, the slope represents surface sediment respiration (milligrams O_2 per square metre per day) and the constant term represents the mean respiration rate in the water column, since when SS/SV tends to 0, sediment respiration is negligible. Below 30 m, water column respiration accounted for 47% of the total respiration whereas below 60 m, it only accounted for 18%. Assuming that the relationship could be extended to the whole lake, the mean respiration rate of the sediment would be about 2.7 mg $O_2 \cdot m^{-3} \cdot d^{-1}$. As the observed mean respiration rate was 6.3 mg $O_2 \cdot m^{-3} \cdot d^{-1}$, this means that about 60% of the respiration took place in the water column;

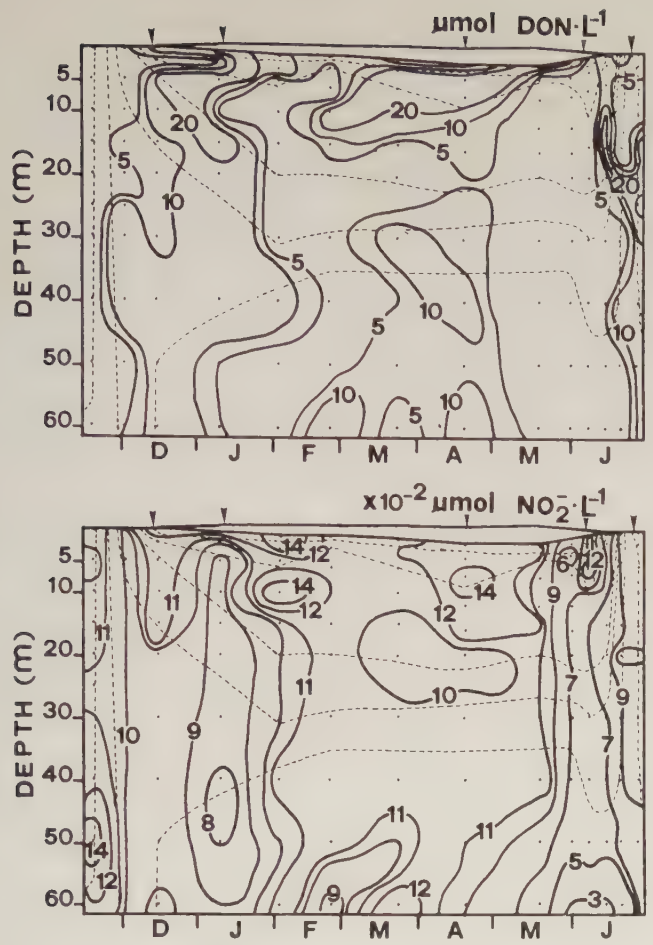

FIG. 10. DON and NO_2^- isopleths for the ice-covered period in Lake Redó, 1984–85. Cover events (arrowheads) and isotherms (broken lines) are shown as in Fig. 2.

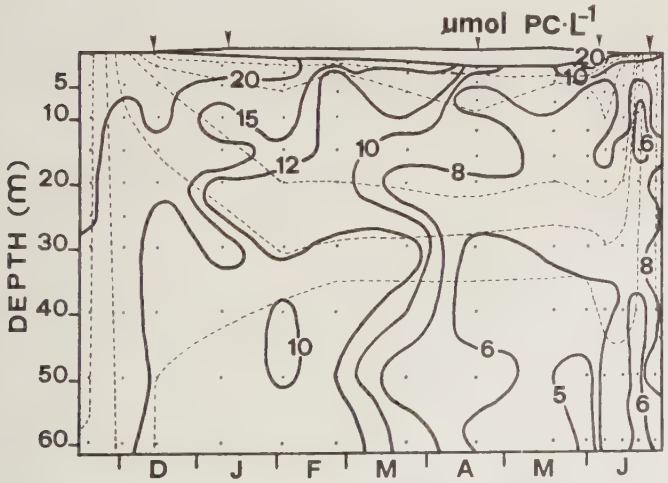

FIG. 11. PC isopleths for the ice-covered period in Lake Redó, 1984–85. Cover events (arrowheads) and isotherms (broken lines) are shown as in Fig. 2.

which is in agreement with the observations in deep, temperate, oligotrophic lakes (Cornett, cited in Welch and Bergmann 1985b).

From mid-January to June, decrease of lakewide mean Chl (milligrams per square metre) fitted a negative exponential

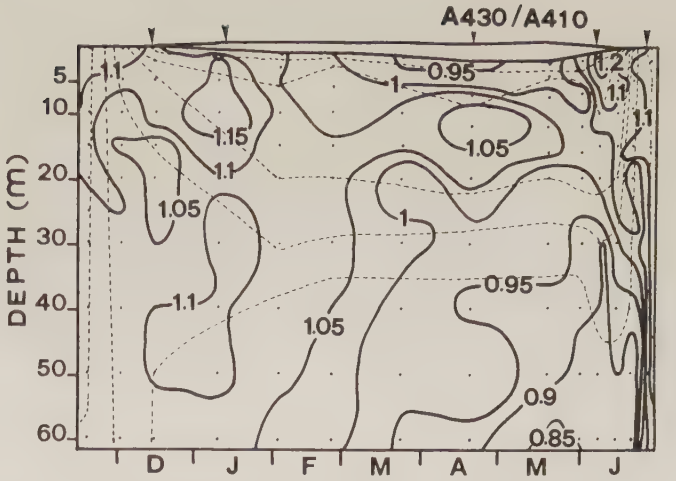

FIG. 12. A430/A410 ratio isopleths of absorbances of pigment extracts during the ice-covered period in Lake Redó, 1984–85. Cover events (arrowheads) and isotherms (broken lines) are shown as in Fig. 2.

curve (Fig. 7):

$$\mathrm{Chl}_t = \mathrm{Chl}_0\, e^{-0.0011t}$$

$$(r^2 = 0.99,\ p << 0.001,\ n = 8)$$

where t is time (days). Until the end of February, Chl was concentrated in the upper 10 m, with a peak between 2 and 5 m, reaching a higher concentration in this layer than in any one during the fall isothermy (Fig. 3). When snow stopped light penetration, the Chl maximum slowly moved down, with smoother and deeper peaks as winter advanced. The deepening of the Chl maximum during the very low light period without outflow, from 3 February to 20 May, had a velocity of 0.14 $m \cdot d^{-1}$, estimated by linear regression of the peak position against time ($r^2 = 0.93$, $p < 0.01$, $n = 5$). Assuming a similar mean sedimentation rate for the phytoplankton of the whole column on those time scales, a specific sedimentation rate of 0.004·d^{-1} can be estimated by dividing the deepening velocity of the Chl peak by the mean depth of the lake (32 m).

During the same period, PC, PN, and PP showed an exponential decrease in concentration, as did Chl (Fig. 7), but their rates of decrease were slower than that of Chl. The three components had similar decay rates (0.005·d^{-1}), with similar significance ($r^2 = 0.83$, $p < 0.01$, $n = 8$). This means a half-life of 4.5 mo for particulate matter. As the phytoplankton sedimentation rate was similar to the decay rate of seston (C, N, and P), it is concluded that most of the particulate organic matter disappeared from the system by sedimentation. The estimated settling velocity (0.14 $m \cdot d^{-1}$) was low, but agrees with the small size of phytoplankton cells (<5 µm) (Catalan and Camarero 1991) persisting in midwinter (Hawley 1982). At the beginning of the winter, after the lake froze, larger particles probably settled much more quickly, explaining the PC:PN:PP changes in the water column. Fluxes of matter to the sediment were 230 mmol $C \cdot m^{-2}$, 26 mmol $N \cdot m^{-2}$, and 1.54 mmol $P \cdot m^{-2}$ from mid-January to June 1985. These figures are similar to those of arctic lakes (Whalen and Cornwell 1985).

The estimated specific sedimentation rate (0.004·d^{-1}) accounts for only 40% of the Chl loss. Therefore, rates of other processes affecting pigment decay (grazing, physiological adaptation, cell senescence, and death) must account for the

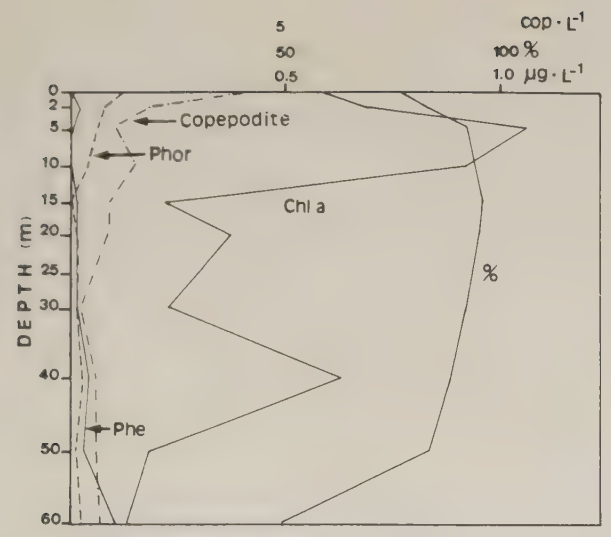

FIG. 13. Vertical profiles of Chl *a*, phaeophorbide *a* (Phor), phaeophytin (Phe), % of nondegradated Chl *a* related pigments, and copepodites of *Cyclops abyssorum* in lake Redó on 20 May 1985.

rest ($0.007 \cdot d^{-1}$). Most of the Chl decrease not accountable to sedimentation may have been due to grazing because dark degradation of pigments seems to occur at a slower rate than degradation following grazing (Welschmeyer and Lorenzen 1985). The estimated grazing rate is rather low (Burkill et al. 1987), probably due to the absence of large zooplankton (adult copepods and cladocerans) from the open water at the time (Catalan and Camarero 1991).

The relative importance of Chl degradation products increased during winter. The A430/A410 ratio showed two points where chlorophyll degradation products accumulated conspicuously (Fig. 12). HPLC pigment determination on 20 May, at the end of this central period, revealed that phaeophorbides predominated near the surface and phaeophytin in the deepest part (Fig. 13). Grazing is the major source of phaeophorbide, while phaeophytin can be a product of several processes (Carpenter et al. 1986). It should be stressed that, even at the end of the central dark period, Chl was the most abundant pigment throughout the column (Fig. 12); only at 60 m did phaeopigment take over (Fig. 12, 13).

Although the physical environment was apparently fairly constant throughout the water column during the central winter period, the top layer was affected by flooding processes and changes in the transparency of the snow cover. Flooding consisted of a flow of water from the lake to the cover to compensate for the hydrostatic imbalance produced by snow accumulation (Jones and Ouellet 1983; Catalan 1989). During that winter, 320 000 m^3 of water from the lake flooded the ice cover from below through cracks, which represented about 1.5 m of water, a mean of 1.2 $cm \cdot d^{-1}$ from mid-January to May. During this period, Na^+ and K^+ showed a clear increase in the upper 5 m (Fig. 14). The most probable sources of these cations were the slush layers of the cover which were richer in salts than lake water because of the composition of some snowfalls with high marine influence (Catalan 1989). If cations from slush entered the lake, other substances and gases also could have penetrated.

There were changes in the transparency of the cover. In a Mediterranean climate, storms alternate with periods of high radiation during winter. On clear days, snow partially melts

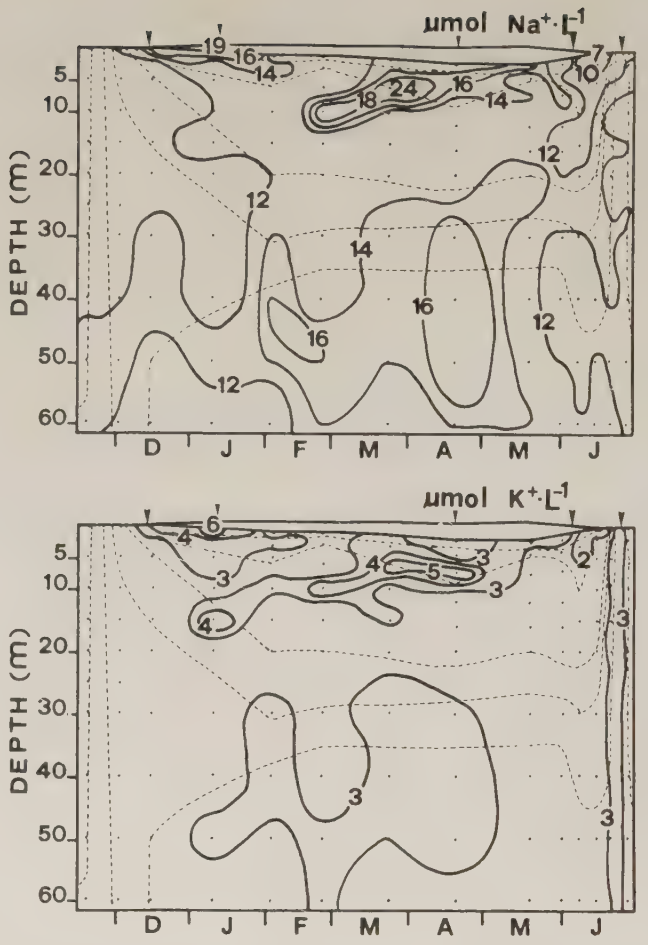

FIG. 14. Na^+ and K^+ isopleths for the ice-covered period in Lake Redó, 1984–85. Cover events (arrowheads) and isotherms (broken lines) are shown as in Fig. 2.

and condenses, and transparency of the cover increases. Two relevant regressive intervals occurred during this central period (Catalan 1989): one at the beginning of April and the other at the end of May, the latter being the start of the thaw.

Until the first important regressive event, the same trends detected in February indicating catabolic processes continued (SRP and DON increased and A430/A410 decreased). Afterward, some changes in the chemical variables studied indicated low photosynthetic activity in the upper layer, although there was no evidence of increased Chl or seston: (a) SRP dropped from 0.1 to 0.03 $\mu mol \cdot L^{-1}$ (Fig. 8), (b) there was a slight increase in O_2 (Fig. 4), and (c) some ratios of seston composition indicated active photosynthesis (Chl *a*:(Chl *b* + Chl *c*) increase and PC:PP decrease). There was also an increase in NO_2^-, which suggested either low light activity of primary producers (Mortonson and Brooks 1980; French et al. 1983) or bacterial activity (McCarthy 1980). Phytoplankton photosynthesizing at very low light levels (<0.1% of incident radiation) have been reported from high-mountain and polar lakes (Tilzer and Schwartz 1976; Vincent 1981). Calculations employing extinction coefficients for snow and ice and considering the cover structure found during sampling (Catalan 1989) gave a maximum light level just underneath the ice of 0.3% of incident radiation on 19 April (Catalan 1988). Direct measurements always showed values of PAR between 0.01 and 1 $\mu E \cdot m^{-2} \cdot m^{-2} \cdot s^{-1}$.

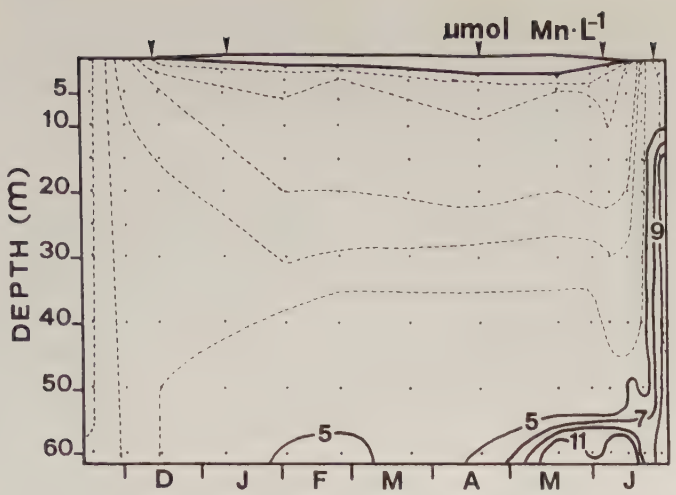

FIG. 15. Mn isopleths for the ice-covered period in Lake Redó, 1984–85. Cover events (arrowheads) and isotherms (broken lines) are shown as in Fig. 2.

The zone between 15 and 30–40 m depth was characterized by the small amplitude of the changes that took place in it. All variables stayed fairly constant, with concentrations tending towards low values. PC, PN, and PP were lower than in the rest of the column. This pattern disappeared at the end of March, at a time when seston concentration decreased steadily from the surface to the bottom. An appreciably lower PC:Chl ratio in this zone than in neighbouring zones above and below also persisted from January (15–30 m) to late April (12–25 m). Concentrations of SRP, dissolved organic substances (SNRP and DON), and the Chl *a*:(Chl *b* + Chl *c*) ratio decreased between the upper and lower parts of the layer. The greatest changes in this zone were in nitrogen compounds. In March, there was a large increase in PN relative to PC and PP (Fig. 7) together with an increase in DON and a decrease in NO_3^- (Fig. 6, 9, 10). The PC:PN ratios (6–5:1) were similar to those characteristic of bacterial populations (Goldman et al. 1987). However, in the following month, NO_3^- increased and particulate matter ratios returned to the usual levels. The increase in PN was associated with an increase in CO_2, while the return to usual values was associated with a decrease in CO_2. Bacterial activities could be responsible for these changes: the former to heterotrophic bacteria using NO_3^- as nitrogen source and the latter to nitrifying bacteria.

Below 30 m, the trend of variables influenced by biological processes was sustained throughout the winter and included a predominance of catabolic processes, with both O_2 (Fig. 4) and pH decreasing. Progressively lower A430/A410 ratios indicated the accumulation of Chl decomposition products (Fig. 12). When heavy snowfalls began, the PC:PN ratio increased quickly to 12, but during the central phase, PC:PN progressively decreased and PN:PP increased. This trend of nitrogen enrichment of seston during the degradation of phytoplankton differs from the observations of Falkowski et al. (1988), who found a relative decrease in nitrogen on marine continental shelves. Our results agree with trends found in the decomposition of leaves in streams (Suberkropp et al. 1976). Whereas DON and NO_2^- decreased here relative to other parts of the water column (Fig. 9), NO_3^- increased, probably as a result of diffusion from the sediment. In contrast, NH_4^+ increased slowly throughout the water column (Fig. 5). There seemed to be a release of phosphorus from the sediment (Fig. 7), as has been observed in other oligotrophic lakes (Kamp-Nielsen 1974), although the minimum pH recorded at 60 m was 6.2 and O_2 saturation was 64%. Simultaneous increase of manganese in the deepest layers also indicates release from the sediment (Fig. 15). The SRP flux was 15.6 µmol $P \cdot m^{-2} \cdot d^{-1}$ from April to May. This flux was not negligible considering the phosphorus limitation of the lake production (Pettersson and Boström 1986). It is difficult to assess the actual sediment area where flux occurred, but it is likely to have been about 15% of the lake area which is at a depth of more than 60 m. On this basis, the actual flux could be 103 µmol $P \cdot m^{-2} \cdot d^{-1}$.

Thaw Period

Continuous melting of the lake cover started in late May and lasted the whole of June (Catalan 1989). In spite of the brevity of this phase, changes in dissolved and particulate matter in the lake were substantial. Some changes in the near-surface water chemistry resulted from the melting of the lake cover and the melting of the watershed snow. At the beginning of the thaw, snowmelt from the catchment entered at a near-surface level and circulated at the surface towards the outlet (Catalan 1989), as happens in other lakes (Hill 1967; Schindler et al. 1974; Hultberg 1977; Bergmann and Welch 1985). There was a peak of inorganic nitrogen compounds (Fig. 6, 10) close to the surface in early June, which could have been the result of the input of water from the cover slush layers which were rich in such compounds when the cover cracked (Catalan 1989). This breaking was followed by a peak of organic matter (Fig. 11), coming from the melting of the cover snow which had been acting as a trap for insects, pollen, and other materials during spring. Much of this floating matter was soon lost through the outflow because of the high surface flow in this period (Catalan 1989). The last melt of the black ice produced decreases in alkalinity and pH to about 10 µeq·L^{-1} and 6.4, respectively, because cover acidity was not compensated for by rock weathering.

At the end of the melting period, there was a slight decrease in the temperature of the water column (Fig. 2) which, although predicted (Ragotzskie 1978; Patterson and Hamblin 1988), has rarely been reported because of the difficulties involved in sampling immediately before and during ice breakup. The time at which the mixed layer reached deep, fine sediments was clearly indicated by a peak of manganese in the water column (Fig. 15).

During thaw, changes in nitrogen forms were marked. NH_4^+ increased in the whole column when the ice cover cracked (Fig. 9), but decreased as the thaw progressed. Although snow was rich in NH_4^+ (Catalan 1989), the increase in the water column was too high (it doubles in 15 d) to be dependent on this source alone; other processes must have been involved to account for such an increase. Parallel to the NH_4^+ increase, there was a marked decrease in DON. Taking into account that accuracy in DON determination is much lower than in NH_4^+ determination, one change could explain the other, although it is difficult to state a mechanism; rate and magnitude seem to be too high to be related to biological activity alone. Reduction of NO_2^- in the water column was also enigmatic. Although there was a decrease by dilution because snow is poor in NO_2^-, in the deepest nonmixed layer, there was a more marked decrease which needs to be explained by another mechanism, in this case, probably biological. These results suggest a need for further research on the nitrogen cycle during thaw.

When the ice cracked at the beginning of June, the increase in Chl and the Chl *a*:(Chl *b* + Chl *c*) ratio showed a rise in primary production. The increase in the A480/A665 ratio from 1.5 to 6 at this time could indicate a physiological response of the low-light-adapted phytoplankton to the suddenly high irradiance. The accompanying increase in SNRP, from 0.15 to 0.25 $\mu mol \cdot L^{-1}$, could have had the same origin.

As the thaw progressed, the Chl peak moved down to 10–20 m. At the middle of the melting period, Chl reached its lowest concentration, although nutrients and light were available. At this time, Chl *a*:(Chl *b* + Chl *c*) was very low (about 15 by weight), contrasting with the higher values in late May (25–30). Dark-adapted phytoplankton populations may not be able to bear high radiation, and a replacement of populations occurred to adapt to the new situation, a process similar to that described in the northern Bothnian Sea by Müller-Haeckel (1985). Kalff and Welch (1974) also found a Chl minimum at the end of the melting period in Char Lake (75°N).

During the whole melting period until the spring isothermy, catabolic processes continued in the deepest layer. The trends described for the central period intensified. For instance, in seston which had been maintaining a PC:Chl ratio of 100 for 5 mo this ratio exceeded 200 in most of the water column and 300 in the deepest part during thaw. These trends ended with the isothermy and a marked increase of Chl.

Conclusions

The ice-covered period cannot be considered as a homogeneous period in all lakes. The physical buildup of the ice and snowpack introduces a temporal partitioning. Snow accumulation is especially important, since significant photosynthetic activity proceeds in lakes with a thick ice cover if the snow is thin (Rodhe 1962; Maeda and Ichimura 1973). In Lake Redó, there are two types of processes during the winter: (a) those which involve larger spatial and temporal scales, related to morphometric and trophic characteristics of the lake, and (b) those which respond to sudden changes of environmental conditions which occur especially near the surface, are ephemeral at a seasonal scale, and seem to depend on the particular behaviour of some organisms or on the adjustment of interactions between components of the planktonic community. Some large-scale processes have been well investigated (lake O_2 consumption and denitrification), but others require more insight (the way in which water and cover interact in terms of fluid motion, solution chemistry, and organism inoculation; nonmonotone vertical distributions during the quiet part of the winter period; composition and reactivity of the DON pool; nutrient diffusion at sediment boundaries in oligotrophic lakes). On the other hand, short time-scale processes occurring under the ice are of current interest both for an understanding of the microbial loop at low temperatures and for the study of the relationship between community structure and physical features.

Acknowledgements

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Ascaridoid Nematodes from the Stomach of Harp Seals, *Phoca groenlandica*, from Newfoundland and Labrador

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Brattey, J., and I-H. Ni. 1992. Ascaridoid nematodes from the stomach of harp seals, *Phoca groenlandica*, from Newfoundland and Labrador. Can. J. Fish. Aquat. Sci. 49: 956–966.

Stomachs of 488 harp seals, *Phoca groenlandica*, collected off Newfoundland and Labrador during 1984–88, contained four species of ascaridoid nematodes. Seals had high prevalences and abundances of *Contracaecum osculatum* B and *Phocascaris* sp. but were rarely infected with *Anisakis simplex* B or *Pseudoterranova decipiens* B (sealworm). Nematode abundance increased with age, and distribution of each species among seals was highly aggregated. Gastric lesions, often associated with clusters of nematodes, occurred in 13.8% of 405 seals. Female seals with large numbers of nematodes had a higher body condition index (sculp weight) than lightly infected females. Overall sex ratios of *C. osculatum* B and *Phocascaris* sp. were biased toward females, but sex ratios of adult nematodes among individual seals were heterogeneous. Most nematodes recovered were third- or fourth-stage larvae; adults of *C. osculatum* B and *Phocascaris* sp. were also common, but no mature females of *A. simplex* B or *P. decipiens* B were found. Harp seals are probably unimportant in the transmission of *A. simplex* but may be partly responsible for sealworm found in fish stocks off Newfoundland and Labrador. More information on other pinniped species is needed to assess more precisely the relative importance of harp seals in sealworm transmission.

Les contenus stomacaux de 488 phoques du Groenland capturés au large de Terre-Neuve et du Labrador entre 1984 et 1988 contenaient quatre espèces de nématodes de la super-famille des Ascaridoidea. La fréquence et l'abondance de *Contracaecum osculatum* B et de *Phocascaris* sp. étaient élevées; par contre, *Anisakis simplex* B et *Pseudoterranova decipiens* B (ver de phoque) étaient rarement présents. Les nématodes, dont le nombre augmentait en fonction de l'âge, formaient des masses dans les phoques infestés. On a observé des lésions gastriques souvent liées à la présence de telles masses chez 13,8 % de 405 phoques. Les femelles infestées d'un grand nombre de nématodes montraient un indice de condition corporelle (poids de la peau et du pannicule) plus élevé que les femelles peu infestées. La proportion relative générale des sexes chez *C. osculatum* B et *Phocascaris* sp. était biaisée en faveur des femelles, mais la proportion relative des sexes des nématodes adultes chez chaque phoque était hétérogène. La plupart des nématodes récupérés étaient des larves de troisième ou de quatrième stade. On retrouvait fréquemment des adultes de *C. osculatum* B et de *Phocascaris* sp., mais aucune femelle mature de *A. simplex* B ou de *P. decipiens* B n'était présente. Le phoque du Groenland ne joue probablement qu'un rôle mineur dans la transmission de *A. simplex*, mais peut être en partie responsable de la présence de vers du phoque chez les stocks de poisson des eaux hauturières de Terre-Neuve et du Labrador. De plus amples données sur d'autres espèces de pinnipèdes sont nécessaires pour évaluer plus précisément le rôle du phoque du Groenland comme vecteur de ce ver.

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The harp seal, *Phoca groenlandica*, is the most abundant pinniped in the Northwest Atlantic, with the population off eastern Canada estimated at 2–2.5 million animals during 1983–85 (Bowen and Sergeant 1985; Malouf 1986). Since the ban on commercial harvesting of white-coat pups in 1983, concerns have been raised about the effects of probable increases in the harp seal population. Commercial fishermen and sealers claim that the growing seal herds have resulted in depletion of fish stocks, damage to fixed fishing gear, and increased numbers of nematode parasites such as sealworm, *Pseudoterranova decipiens*, in the flesh of various fish species.

The grey seal, *Halichoerus grypus*, is generally considered the principal definitive host of sealworm in the North Atlantic (Young 1972; Mansfield and Beck 1977; McClelland 1980a; Stobo et al. 1990), with smaller numbers of *P. decipiens* also occurring in harbour seals, *Phoca vitulina* (McClelland 1980a; Lick 1989). Adult sealworm has also been reported from harp seals off eastern Canada (Lyster 1940; Myers 1957a, 1957b; Scott and Fisher 1958), but the overall importance of harp seals as definitive hosts of sealworm is unclear. Detailed studies of ascaridoid nematodes from harp seals have been primarily confined to the Gulf of St. Lawrence (Scott and Fisher 1958). The study by Scott and Fisher (1958) included 111 harp seals from eastern Newfoundland, but only a small proportion of the nematodes recovered were identified; also, these seals were collected solely during March–May and consisted mainly of animals more than 3 mo old. Little is known about anisakine infections in the large harp seal herd that breeds off southern Labrador and northeast Newfoundland (the "front" herd).

In this paper, we present quantitative information on nematode parasite burdens in 488 harp seal stomachs collected off Labrador and the south, east, and northeast coasts of Newfoundland during 1984–88. The purpose of the study was to investigate the possible role of harp seals in transmitting ascaridoid nematodes, particularly sealworm, to fish stocks off Newfoundland and Labrador.

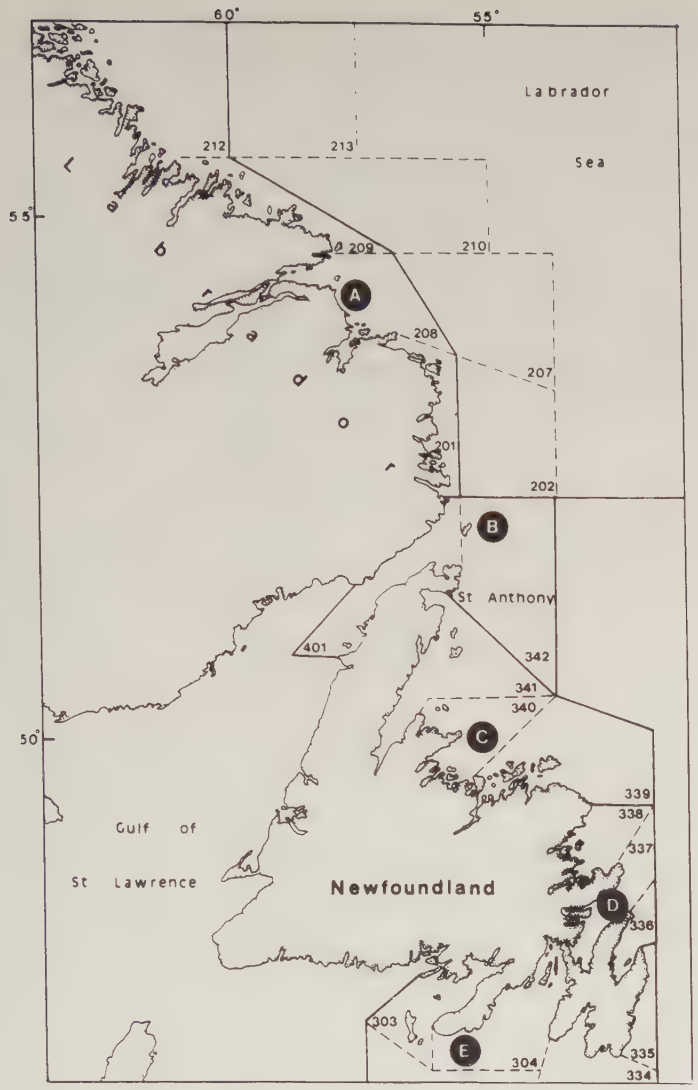

FIG. 1. Eastern Canada showing sampling areas and place names mentioned in the text. Solid lines indicate boundaries of the five general areas sampled; broken lines indicate boundaries of numbered sampling locations within each area. A = Labrador coast, B = St. Anthony, C = northeast coast of Newfoundland, D = east coast of Newfoundland, E = south coast of Newfoundland.

Materials and Methods

Stomachs of harp seals were collected by seal hunters and fishermen at various locations off Labrador and the south, east, and northeast coasts of Newfoundland (Fig. 1) during 1984–88. Stomachs were sealed individually in cloth bags immediately after removal. Approximately 50 stomachs were examined fresh, but most were examined after preservation in 70% ethyl alcohol or after being frozen and stored for 1–5 mo at −20°C before preservation in alcohol. The date, location, and sex of each animal were recorded (Table 1). The lower jaw was removed and a canine tooth extracted; the age of the seal was determined using dental annuli (Bowen et al. 1983) assuming a birth date of March 1. Standard morphological measurements (American Society of Mammalogists 1967) were also recorded from approximately 20% of the seals. Details of the sampling areas, age structure, and collection dates of samples are summarized in Table 1. Stomachs (and storage bags) were slit open and the contents washed into a series of sieves (mesh sizes 2.0 mm and 850, 500, 250, and 100 μm). Nematodes were removed from semidigested food items and from the gastric mucosa with forceps. The presence or absence of ulcerative lesions on the stomach wall was noted. Initially, nematodes were roughly sorted by eye into four length categories (approximate sizes <1, 1–1.9, 2–4, and >4 cm) and preserved in a 1:9 solution of glycerol in 70% ethyl alcohol; hot solution (70°C) was used for fresh specimens. Nematodes were cleared in glycerin or lactic acid and examined under a compound microscope (× 100). Criteria for distinguishing the various species and stages of nematodes encountered were obtained from the literature (e.g. Lyster 1940; Berland 1961, 1963; Davey 1971; McClelland 1980a). The specimens were identified and categorized as third- or fourth-stage larvae (L3's or L4's), adult males, immature adult females (with no eggs in the uterus), or mature adult females (with fully developed eggs in the uterus). In addition, specimens were sent to Prof. L. Paggi and coworkers at the University of Rome "La Sapienza," Rome, Italy, to confirm the identifications by means of allozyme electrophoresis (see Discussion).

In approximately 25% of the stomachs, nematodes in the two smaller size categories were too numerous to count and identify individually; therefore, the numbers of each species and stage in these size categories were determined by subsampling using a 10-chambered "whirling" apparatus (see Pitt 1965). To determine whether the apparatus would produce random subsamples of the total number of small nematodes, we placed known numbers of nematodes into the apparatus and counted the numbers recovered from each chamber. The mean number per chamber and variance was calculated, and a variance to mean ratio test (Elliott 1979) was used to test for randomness. In 20 trials, chi-square values ranged from 4.0 to 16.0 (with 9 df), indicating good agreement with a random distribution. Subsequently, we added all the nematodes from one size group to the apparatus, counted and identified the nematodes recovered from three randomly selected chambers, and estimated the totals of each species and stage by multiplication. The subsampling technique was not suitable for larger nematodes which tended to cluster together; consequently, all nematodes in the two larger size categories (2–4 and >4 cm) were cleared and examined under a compound microscope. Total counts of each species and stage were obtained by pooling results from subsampling of the smaller size groups with results obtained from individual examinations of larger specimens.

Statistics

The terms prevalence (percent infected) and abundance (mean number of parasites per seal) follow standard usage (Margolis et al. 1982). The significance of differences in these infection statistics was tested by *G*-tests and nonparametric Kruskal–Wallis (or Mann–Whitney) tests, respectively. Various transformations were attempted to remove heteroscedasticity and normalize the distribution of nematode counts, but none was successful. The dispersion pattern of each nematode species among seals was investigated initially by chi-square tests of the ratio of the mean number of nematodes per seal to the sample variance. Observed frequencies of nematodes were tested for goodness-of-fit to three discrete two-parameter distributions using maximum likelihood methods (Ross 1980). These distributions, Polya–Aeppli, negative binomial, and Poisson lognormal, differ in their degree of relative skewness, the Polya-Aeppli being the least skewed and the Poisson log-

TABLE 1. Sampling areas, ages, and collection dates for harp seal stomachs examined for ascaridoid nematodes. See Fig. 1 for boundaries of sampling areas and location codes.

Sampling area	Location code	Age class (yr)							Collection dates
		0	1	2	3	4	≥5	Unknown	
A. Labrador coast	212	—	2	1	3	1	—	—	Nov.–Dec. 1986; Nov. 1988
	209	—	2	6	14	5	9	1	Nov.–Dec. 1984; Nov.–Dec. 1985; Dec. 1986
	201	—	7	10	14	4	9	2	Nov.–Dec. 1984; Dec. 1986
B. St. Anthony	401	—	15	10	8	2	3	—	Dec. 1984; Dec. 1985; Nov.–Dec. 1986
	342	15	12	21	14	6	39	—	Dec. 1984; Feb., May–June 1985; Jan.–May, Dec. 1986; Jan.–Feb. 1987
C. Northeast coast of Newfoundland	341	—	2	4	8	5	13	1	Feb. 1985; Feb. 1986; Jan. 1987
	340	—	8	8	4	2	3	—	Jan.–Mar. 1986
	339	3	5	4	5	1	—	—	May 1985; Mar.–May 1986
D. East coast of Newfoundland	338	8	7	—	—	—	—	—	May 1985; Mar.–Apr. 1986
	337	40	1	—	—	—	—	—	May 1985
	336	23	6	4	2	—	—	—	May 1985
E. South coast of Newfoundland	335	1	—	—	—	—	—	—	June 1987
	334	26	12	10	5	1			June–July 1987
	304	17	9	3	1	—	—	—	June 1987
	303	—	1	—	—	—	—	—	June 1987

normal the most skewed. Cells in the distribution of each species were grouped in a consistent manner to minimize the frequency of zero counts and to permit comparison between age groups. Relative goodness-of-fit to each model is judged by comparing the values of chi-square.

Nematode Abundance and Seal Body Condition

Variation in nematode abundance with seal body condition was also investigated. Sculp weight (= weight of blubber with epidermis) was used as an index of body condition. To permit comparison of body condition among seals of a wide range of sizes, variation in body size (standard length, SL) was removed from the measure of body condition using methods described by Read (1990). SL^3 rather than SL was used to correct for variation in sculp weight with size in order to maintain dimensional similarity (Stahl 1962). Least squares techniques were used to generate linear regressions of sculp weight on SL^3. The resulting residuals represent a measure of condition after the effect of body size has been removed. Residuals were generated using the REG procedure of SAS (SAS Institute Inc. 1988).

Seals were divided into three categories according to the number of nematodes in the stomach: (1) 1–600, (2) 601–1200, and (3) >1200. To eliminate possible sex, seasonal, and annual effects the analyses were conducted separately for male and female seals and were based solely on animals collected during June and July 1987. Mean residual values among seals in each category were compared using the general linear models procedure of SAS; Bonferroni *t*-tests and Tukey's test were used to investigate the significance of differences among group means.

Results

Approximately 419 000 nematodes were recovered from the stomachs of 488 harp seals. Only three stomachs were free of nematodes and most (67%) contained more than 300. Twenty-one animals had more than 3000 nematodes, the maximum number being 9637 worms. The nematodes belong to four ascaridoid genera, namely *Anisakis*, *Contracaecum*, *Phocascaris*, and *Pseudoterranova* (Table 2).

Most of the nematodes recovered from harp seals were L3's or L4's (Table 2). Although adults of each species were also found, adult *Anisakis* and *Pseudoterranova* were rare. *Contracaecum osculatum* B was clearly the most numerous nematode; only three harp seals were not infected with this species and the overall abundance was 642.4. Infections with each nematode species were characterized by variance to mean ratios greater than 1, indicating that the distribution of each nematode species was highly aggregated. Maximum numbers of each nematode species ranged from 50 *P. decipiens* to 4453 *C. osculatum* B. There were no significant differences between male and female seals with regard to prevalence ($0.9 > P > 0.1$, df = 1) or abundance ($0.9 > P > 0.1$) of each nematode species.

Geographic Differences

There was considerable geographic heterogeneity in the nematode parasite infections of harp seals (Table 2). *Contracaecum osculatum* B was clearly the most prevalent nematode (100% in most areas). The abundance of *C. osculatum* B differed significantly among sampling areas ($P < 0.0001$, df = 4), ranging from 246.9 in Labrador to 1152.3 in samples collected off the south coast of Newfoundland. Adult *C. osculatum* B, including mature females, was observed in harp seals in all areas, but there were geographic differences in the population structure of this nematode. The proportion of adult nematodes varied inversely with abundance (Table 2), and proportions of adult *C. osculatum* B in harp seals from more northerly areas of Labrador, St. Anthony, and the northeast coast were higher (26.8–64.2%) than those from the south and east coasts (9.6–17.8%).

Phocascaris sp. was also common in harp seal stomachs, with prevalences ranging from 36.6 to 60.5% (Table 2). Abundances of *Phocascaris* differed significantly among areas ($P < 0.0001$, df = 4) and were highest off the northeast coast (139.2 nematodes per seal) but low elsewhere (12.2–29.6).

TABLE 2. Geographic variation in the prevalence, abundance, and population structure of ascaridoid nematodes from the stomach of harp seals collected off Newfoundland and Labrador during 1984–88. Prevalence = % infected, abundance = mean numbers of nematodes per seal including uninfected seals, SE = standard error, n = sample size.

Species	Statistic	Geographic area				
		A. Labrador (n = 90)	B. St. Anthony (n = 145)	C. Northeast coast of Newfoundland (n = 76)	D. East coast of Newfoundland (n = 91)	E. South coast of Newfoundland (n = 86)
Anisakis simplex (Type B)	Prevalence	1.1	2.8	6.6	16.5	14.0
	Abundance ± SE	0.01	0.01 ± 0.07	1.5 ± 1.09	2.0 ± 0.83	3.2 ± 1.19
	Variance:mean ratio	—	7.35	60.15	31.59	38.62
	Maximum infection	1	10	76	58	75
	% third-stage larvae	—	100.0	100.0	83.3	82.7
	% fourth-stage larvae	—	—	—	16.7	17.3
	% adult males	—	—	—	—	—
	% adult females (mature)	100 (—)	— (—)	— (—)	— (—)	— (—)
	% unknown stage	—	—	—	—	—
Contracaecum osculatum (Type B)	Prevalence	100.0	100.0	96.1	98.9	100.0
	Abundance ± SE	246.9 ± 24.90	694.3 ± 76.56	772.9 ± 102.71	376.3 ± 43.86	1152.3 ± 93.72
	Variance:mean ratio	225.97	1224.07	1037.35	465.18	655.52
	Maximum infection	1182	4380	4453	1941	3515
	% third-stage larvae	1.2	2.6	10.9	2.7	18.6
	% fourth-stage larvae	27.8	57.5	43.7	65.0	65.9
	% adult males	23.5	9.3	11.0	4.3	3.5
	% adult females (mature)	40.7 (11.0)	17.5 (2.8)	24.9 (4.9)	13.5 (1.0)	6.1 (1.0)
	% unknown stage	6.9	13.0	9.3	14.5	5.8
Phocascaris sp.	Prevalance	53.3	36.6	57.9	41.8	60.5
	Abundance ± SE	12.2 ± 3.10	18.2 ± 6.58	139.2 ± 44.27	21.8 ± 7.18	29.6 ± 6.71
	Variance:mean ratio	71.17	345.63	1069.92	215.12	130.91
	Maximum infection	172	871	2262	449	397
	% third-stage larvae	24.5	29.7	35.6	57.9	60.5
	% fourth-stage larvae	19.6	46.4	46.8	25.4	36.9
	% adult males	18.1	7.6	3.2	0.6	1.1
	% adult females (mature)	30.0 (19.7)	11.6 (7.6)	7.2 (2.4)	5.5 (0.2)	1.3 (0.8)
	% unknown stage	7.9	4.8	7.2	10.6	0.2
Pseudoterranova decipiens (Type B)	Prevalence	0	0	1.3	1.1	4.7
	Abundance ± SE	—	—	0.7	0.1	0.5 ± 0.30
	Variance:mean ratio	—	—	—	—	15.61
	Maximum infection	—	—	50	10	22
	% third-stage larvae	—	—	80.0	0	23.3
	% fourth-stage larvae	—	—	—	100.0	74.4
	% adult males	—	—	—	—	2.3
	% adult females (mature)	—	—	— (—)	— (—)	— (—)
	% unknown stage	—	—	20.0	—	—

Geographic differences in the population structure of *Phocascaris* were similar to those of *C. osculatum*. In each area, most specimens recovered were L3's and L4's. The proportion of adults tended to decline in a north to south direction, from 48.1% off Labrador to 2.4% off the south coast of Newfoundland (Table 2); this difference, which was also observed for *C. osculatum* (Table 2), can possibly be attributed to heterogeneity in the age composition of the samples. Most of the older seals were collected in the more northerly areas (Table 1), and older animals tended to have higher proportions of adult nematodes (see below). Some *Phocascaris* sp., particularly the largest adult specimens, occur in the anterior intestine of seal hosts. Since intestines were not available for examination, the data presented here are based on stomach burdens alone and therefore underestimate the total number of *Phocascaris* sp. present in harp seals.

Although *Anisakis simplex* B was found in harp seals in all areas, prevalences differed significantly ($P < 0.001$) and were higher in samples from the south and east coasts (14.0–16.5%) than elsewhere (1.1–6.6%). Abundances of *A. simplex* A were generally low (0.01–3.2), and with the exception of a single immature adult female, all specimens recovered were L3's or L4's.

Pseudoterranova decipiens B was found in harp seals from only three areas, the prevalence being greatest (4.7%) in seals from the south coast of Newfoundland. Most infections consisted of small numbers (<50) of L3's and L4's. No adult females and only one adult male were recovered.

Effect of Host Age

Almost all (98–100%) harp seal pups collected during the first 2 wk of May were infected with *C. osculatum* B (Fig. 2). The prevalence of *C. osculatum* B remained close to 100% throughout May and June; abundance increased significantly ($P < 0.0001$, df = 3) with time from 221.0 to 824.7 during this period (Fig. 2). Most specimens of *C. osculatum* B from pups were L3's or L4's, but the proportion of L3's appeared to

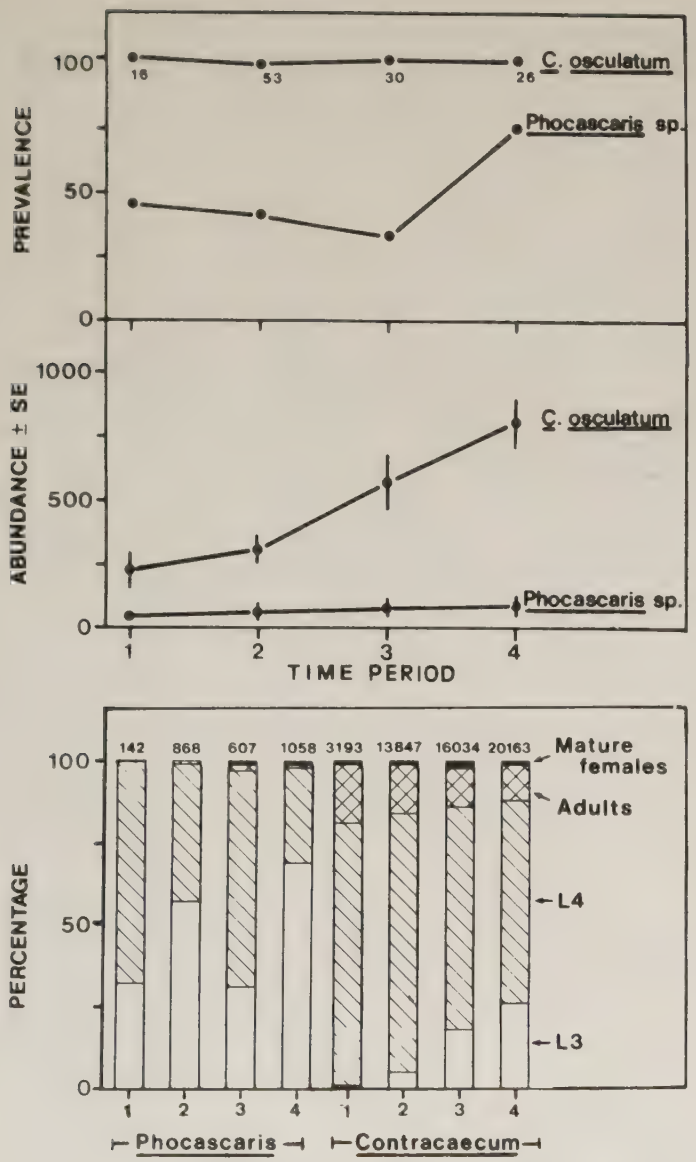

FIG. 2. Temporal changes in the prevalence (% infected), abundance (mean ± SE), and population structure of *C. osculatum* B and *Phocascaris* sp. from the stomach of harp seal pups collected off Newfoundland during May–June. Numbers on each graph or histogram are sample sizes. Time periods (horizontal axis): 1 = May 1–15, 2 = May 16–31, 3 = June 1–15, 4 = June 16–30.

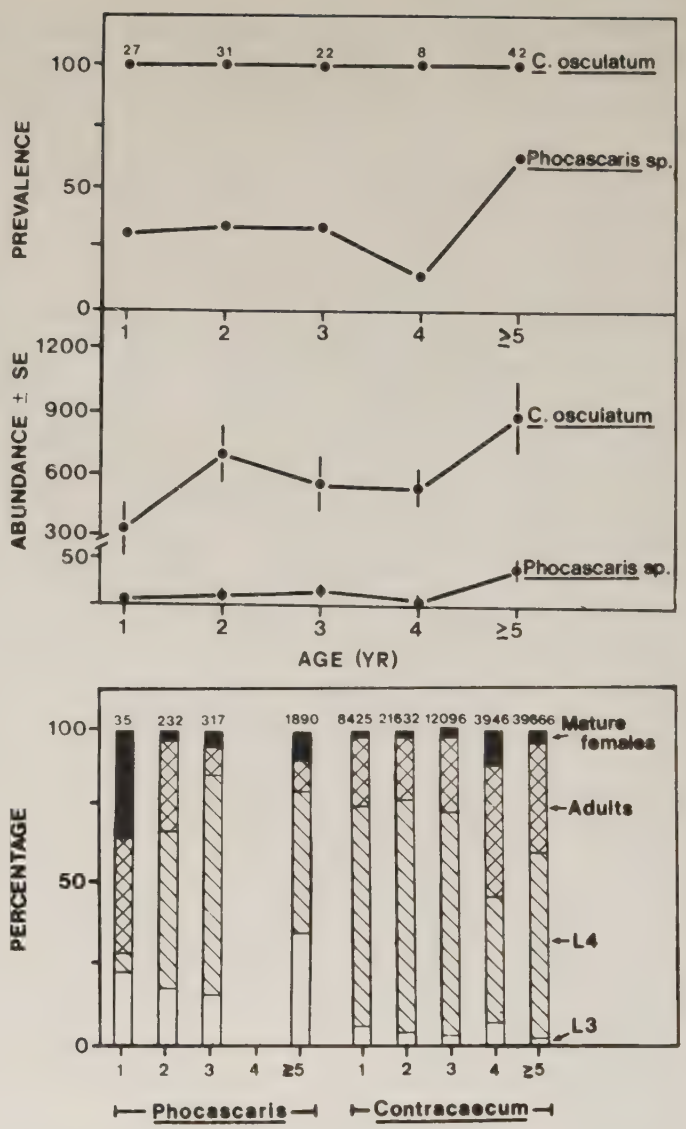

FIG. 3. Host age-related changes in the prevalence (% infected), abundance (mean ± SE), and population structure of *C. osculatum* B and *Phocascaris* sp. from the stomach of harp seals collected off St. Anthony, Newfoundland. Numbers on each graph or histogram are sample sizes.

increase through May and June (Fig. 2). Adult worms were also found during this period, although the proportion of mature females was consistently low (0.3–1.4%).

Phocascaris sp. was far less prevalent (25–73.1%) than *C. osculatum* B in the stomachs of pups collected in May and June (Fig. 2). There were significant increases in the prevalence ($P < 0.01$) and abundance ($P < 0.002$) of *Phocascaris* sp. during the 2-mo period, with the infection level reaching 73.1% and 40.7, respectively, by late June. The population structure of *Phocascaris* sp. showed no distinct changes with time; most of the specimens recovered were L3's and L4's (Fig. 2). Small proportions of adult *Phocascaris* (1.2–2.6%) were also found in the stomach of pups collected during the 2-mo period, although mature females were not observed until late May.

To eliminate possible geographic effects, the relationship between host age and nematode infection among older seals (i.e. ≥1 yr) was investigated using samples collected from one area, namely St. Anthony. In these seals the prevalence of *C. osculatum* B was 100% in all age groups (Fig. 3). The abundance of *C. osculatum* B varied significantly among age groups ($P < 0.002$) and appeared to increase slightly with age (Fig. 3). There were no distinct host age-related changes in the population structure of *C. osculatum*; the values for 4-yr-old seals are probably anomalous due to the small host sample size ($n = 8$). Usually, L3's and L4's were the most numerous stages recovered from each host age group, but adult worms, including mature females, comprised 22.2–52.8% of the totals.

The prevalence and abundance of *Phocascaris* sp. in the stomach of older (≥1 yr) harp seals from St. Anthony differed significantly with age ($P < 0.02$ and $P < 0.003$, df = 4), and age-related trends were similar to those of *C. osculatum* B (Fig. 3); however, this species was much less common in the stomach than *C. osculatum* B. The proportions of the various stages of *Phocascaris* sp. appeared variable (Fig. 3), but the value for 1-yr-old seals is based on a small number of worms

(35) and may not be representative; also, only two specimens of *Phocascaris* sp. were recovered from 4-yr-old seals; therefore, the nematode population structure in this seal age group could not be determined. Among the remaining age groups, no distinct age-related trends were evident; L3's and L4's were most numerous, but adults, including mature females, comprised 13.8–32.3% of the totals. The proportions of adult *C. osculatum* B and *Phocascaris* sp. were notably greater in older seals compared with pups (Fig. 2 and 3).

Nematode Frequency Distributions

To investigate host age-related changes in the frequency distribution of *C. osculatum* B, seals were grouped into four age categories: <1, 1–2, 3–4, and ≥5 yr. For each species the data for all stages (L3's, L4's, and adults) and for mature females were fitted separately to three two-parameter distributions (Table 3). The distributions were best described by the negative binomial model. The values of μ (mean) tended to increase, reflecting the host age-related increase in the abundance of *C. osculatum* B. Values of the parameter k of the negative binomial were below 1, indicating a high degree of aggregation. There were no distinct age-related trends in the value of k for the fits to data on all stages, but values of k tended to increase with host age for the fits to data on mature females only. These results suggest that the degree of aggregation of mature females, as measured by k, tended to decline with host age.

Nematode Sex Ratios

The overall sex ratios of adult *C. osculatum* B (n = 78 766) and *Phocascaris* sp. (n = 2320) from harp seals differed significantly from 1:1 (G-tests, $P < 0.0001$, df = 1), with females outnumbering males of each species by approximately 2:1. An overall bias in the sex ratio towards females was evident for both nematode species in all months sampled (November–July).

Variation in the sex ratio of *C. osculatum* B among individual seals was investigated using replicated goodness-of-fit tests (G-statistic) assuming an expected male to female ratio of 1:2. Sex ratios of *Phocascaris* sp. were omitted from further analyses because adults from the anterior intestine were not available for examination. Seals with less than five adult *C. osculatum* B of each sex were omitted from these analyses to eliminate problems associated with low cell frequencies (Sokal and Rohlf 1981). The sex ratios of *C. osculatum* B among individual seals were markedly heterogeneous (G = 7760.7, $P < 0.0001$, df = 344). The pooled data (all samples combined) were used to test the hypothesis that the overall sex ratio (males to females) was 1:2; the hypothesis was rejected (G = 53.9, $P < 0.0001$, df = 1). Values for total G (i.e. pooled G plus heterogeneity G) indicated that the data as a whole did not fit a 1:2 sex ratio (G = 7814.5, $P < 0.0001$, df = 343). Clearly, this was due to the significant heterogeneity in the sex ratios of *C. osculatum* B among individual seals. Sex ratios of *C. osculatum* B and *Phocascaris* sp. among individual harp seals were highly variable, with strong biases towards either sex in some animals (Fig. 4).

Gastric Lesions

Large, crater-like ulcers were observed in the stomach wall of 13.8% of 405 harp seals. Clusters of *C. osculatum* B and *Phocascaris* sp. at various stages of development (L3, L4, immature adult) often protruded from the centre of the ulcer, but lesions also occurred in stomachs containing few worms. The nematodes were often firmly anchored in the depression in the centre of the lesion and could not be extracted without sustaining damage; the anterior of many nematodes appeared to be surrounded by a "hyaline" cap. Adult *Phocascaris* sp. were often scattered around the fundic region of the stomach whereas those of *C. osculatum* B tended to be more clustered. The prevalence of lesions did not vary significantly with host sex ($P > 0.3$, df = 1) or age ($P > 0.8$, df = 5), but lesions were more prevalent in seals from the northeast (29.3%) and east coasts (28.9%) of Newfoundland compared with those from Labrador (1.1%) and St. Anthony (4.8%) (lesions were not recorded in samples collected off the south coast). There were no significant differences in the prevalence of *C. osculatum* B ($P > 0.5$, df = 1) or *Phocascaris* sp. ($P > 0.19$, df = 1) between seals with or without lesions; however, both nematodes were significantly more abundant ($P < 0.001$ and $P < 0.02$, df = 1) in seals with lesions (means were 754.8 for *C. osculatum* B and 71.0 for *Phocascaris* sp.) than in seals with no lesions (means of 500.7 and 35.5, respectively).

Nematode Abundance and Seal Body Condition

Sculp weight increased with SL^3 for both male and female seals in each nematode abundance category, and the largest individuals usually had the highest sculp weights (Fig. 5). Since nematode abundance tended to increase with seal age, more of the larger seals were in the highest nematode abundance category. There were significant linear relationships between sculp weight and SL^3 in each category for females ($7.1 > t > 4.1$,

TABLE 3. Variance to mean ratios and maximum likelihood estimates for parameters of various frequency distribution models fitted to data on *C. osculatum* B in various age groups of harp seals from Newfoundland and Labrador. Relative goodness-of-fit to each model is judged by values of the chi-square statistic (χ^2).

	Age group (yr)	Number of seals	Variance to mean ratio	Degrees of freedom	Polya–Aeppli			Negative binomial			Poisson lognormal		
					M1	Q	χ^2	μ	k	χ^2	M	σ	χ^2
C. osculatum (all stages)	0	133	544.17	15	2.63	0.99	59.7	464.61	0.75	20.8	5.63	1.35	82.4
	1–2	170	948.09	17	2.40	1.00	103.0	689.60	0.61	12.6	5.85	1.55	91.7
	3–4	105	799.39	17	2.50	1.00	51.1	551.18	0.81	16.5	5.77	1.22	41.5
	≥5	76	1400.02	17	2.28	1.00	69.3	1017.30	0.65	34.4	6.19	1.45	54.3
C. osculatum (mature females only)	0	133	27.05	21	0.55	0.87	25.1	4.33	0.18	13.8	−0.56	2.12	210.4
	1–2	170	24.33	20	1.41	0.90	57.4	13.92	0.46	35.4	41.72	1.67	275.8
	3–4	105	46.52	25	1.81	0.95	68.1	33.79	0.59	40.3	2.88	1.70	170.2
	≥5	76	222.12	25	1.91	0.96	55.9	47.66	0.57	37.9	3.42	1.80	116.1

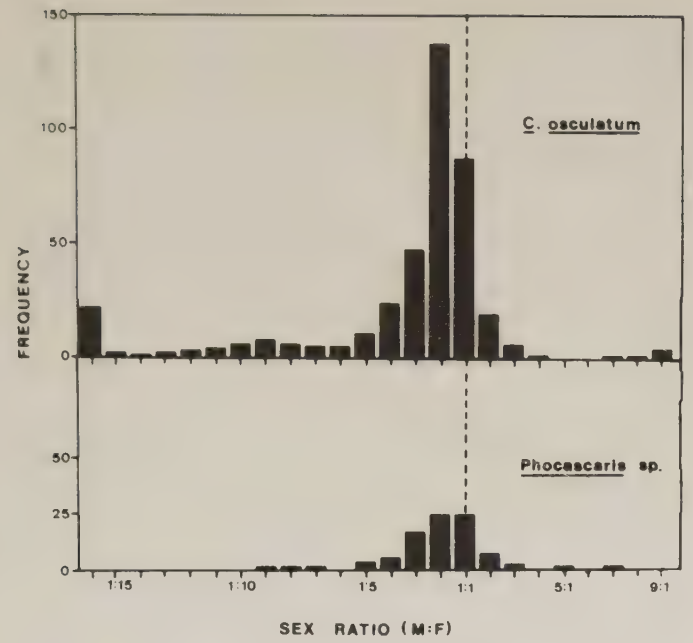

FIG. 4. Sex ratios of adult *C. osculatum* B and *Phocascaris* sp. from the stomach of harp seals from Newfoundland and Labrador.

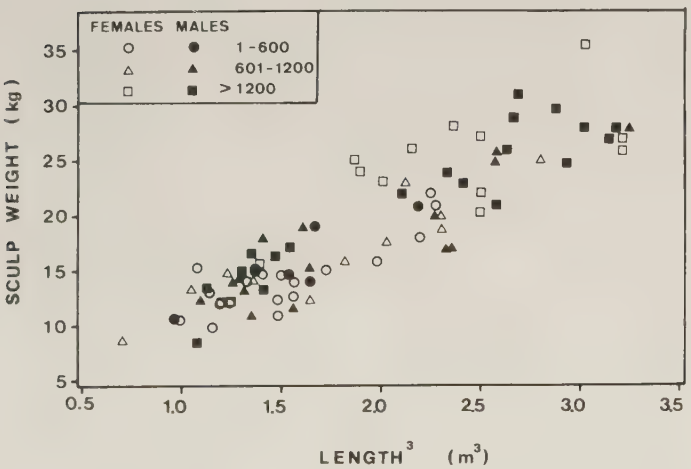

FIG. 5. Relationship between sculp weight and body size in male and female harp seals infected with different numbers of ascaridoid nematodes. The seals were collected off Newfoundland and Labrador during June and July 1987.

TABLE 4. Variation in residuals of sculp weight in male and female harp seals from Newfoundland and Labrador in relation to the numbers of ascaridoid nematodes in the stomach. Means within each sex followed by the same letter are not significantly different ($p > 0.05$) according to Bonferroni *t*-tests.

Host sex	Nematode abundance	*n*	Mean ± SD
Females	0–600	19	−0.98 ± 1.85 a
	601–1200	12	−0.42 ± 2.07 ab
	>1200	15	1.57 ± 3.99 b
Males	0–600	6	−0.15 ± 1.63 a
	601–1200	15	−0.86 ± 2.73 a
	>1200	19	0.73 ± 2.45 a

$0.0001 < P < 0.001$, df = 1) and males ($10.3 > t > 4.1$, $0.0001 < P < 0.01$, df = 1). ANCOVA revealed no difference in slopes between the regression lines for females ($F = 0.02$, $P = 0.98$, df = 2) or males ($F = 0.35$, $P = 0.71$, df = 2). Residuals from the common regression line for each sex represent size-converted measures of body condition. Body condition (as measured by residuals of sculp weight) appeared to increase with the number of nematodes present in female seals only (Table 4); ANOVA confirmed that the residuals of sculp weight varied significantly among nematode abundance categories for female seals ($F = 3.73$, $P = 0.03$, df = 2) but not for males ($F = 1.74$, $P = 0.19$, df = 2). Bonferroni *t*-tests revealed that residuals of sculp weight were significantly different between female seals in nematode abundance categories 1 and 3 (Table 4); Tukey's test gave similar results.

Discussion

Recently, genetic studies based on allozymes have shown that many of the ascaridoid nematode genera infecting marine mammals in the North Atlantic consist of several morphologically similar but genetically distinct species (Orecchia et al. 1986; Nascetti et al. 1986, 1990; Paggi et al. 1991). Since formal morphological descriptions of the various species within each genus have yet to be completed, specific names have not been assigned and the species have provisionally been designated as Types A, B, C, etc. Preliminary genetic analyses of specimens from the seals examined here have revealed that Canadian Atlantic harp seals harbour the following types: *A. simplex* B, *P. decipiens* B, *C. osculatum* B, and *Phocascaris* sp. (see below). Details of the methods used to distinguish these species from their congeners are described elsewhere (Orecchia et al. 1986; Nascetti et al. 1986, 1990; Paggi et al. 1991).

From an applied fisheries perspective, the most significant finding of this study is the rarity of *P. decipiens* (Type B), particularly mature adult females, in the stomach of harp seals from Newfoundland and Labrador. Data presented by Myers (1957b) indicate a prevalence and abundance of 12.3% and 4.38 nematodes per seal for *P. decipiens* in 195 harp seals collected in the Gulf of St. Lawrence near the Magdalen Islands during 1956. Scott and Fisher (1958) found that harp seals were not infected with sealworm when they entered the Gulf during January but acquired the nematode mainly during the postnuptial feeding period; infection levels varied widely between years and with the age of the seal. These results suggest that the two harp seal breeding herds that occur off eastern Canada differ markedly with respect to their level of infection with adults of *P. decipiens*. Harp seals from the Gulf herd acquire considerable numbers of sealworm, some of which develop to maturity; consequently, these harp seals undoubtedly contribute to the infection of fish stocks in the Gulf, which are often heavily infected (Templeman et al. 1957; Wiles 1968; McClelland et al. 1985, 1987). The precise importance of harp seals in the transmission of sealworm to fish in the Gulf relative to that of the much more heavily infected grey and harbour seals, however, remains unknown. Although several attempts have been made to estimate the relative importance of grey, harbour, and harp seals as hosts for *P. decipiens* in the Gulf (Scott and Fisher 1958; Mansfield and Sergeant 1965; Mansfield 1968; Mansfield and Beck 1977), the size of these seal populations has changed dramatically in recent years (Bowen and Sergeant

1985; Stobo and Zwanenburg 1990; Zwanenburg and Bowen 1990), so these estimates are probably no longer valid. The importance of each seal species as a host for *P. decipiens* in the Gulf needs to be reevaluated.

The rarity of adult *P. decipiens* in the "front" herd is consistent with the low numbers of larval sealworm found in Atlantic cod, *Gadus morhua*, inhabiting waters off Labrador and northeast Newfoundland (Brattey et al. 1990). Although the larval sealworm infections in cod in these areas may be sustained by the huge but lightly infected harp seal herd, there are several other species of seal in these waters that may also be important in the transmission of sealworm. In addition to grey and harbour seals, there are populations of bearded, *Erignathus barbatus*, hooded, *Cystophora cristata*, and ringed seals, *Phoca hispida*, which may be infected with *P. decipiens*. Information on population sizes and infection levels of each seal species is needed before an estimate can be made of the relative importance of harp seals in the transmission of sealworm.

This study also indicates that harp seals off Newfoundland and Labrador are not suitable definitive hosts for *A. simplex* B and that these seals are probably unimportant in the transmission of *Anisakis* to fish stocks. Although larvae of *A. simplex* B are not uncommon in harp seals from the south and east coasts of Newfoundland, our results give no indication that the parasite develops to maturity in this pinniped. Adults of *Anisakis* sp. occur mainly in cetaceans (Lyster 1940; Cowan 1963; van Thiel 1966; Young 1972), although McClelland (1980a) and Young (1972) also found mature females in the stomach of grey seals, suggesting that this pinniped may be a secondary definitive host.

Three species of *Phocascaris* have been described from seals, namely *P. phocae* from harp seals in the White Sea (Host 1932, *P. netsiki* from ringed seals in the Canadian Arctic (Lyster 1940), and *P. cystophorae* from hooded seals off northern Norway (Berland 1963). Morphologically, our specimens resemble *P. cystophorae* in that they possess interlabial knobs; however, genetic evidence based on allozymes indicates that the taxonomy of the genus *Phocascaris* infecting seals is confused and in need of revision (Nascetti et al. 1990). Genetic analyses of specimens of *Phocascaris* from Canadian harp, hooded, and ringed seals is currently underway (Prof. L. Paggi, University of Rome, "La Sapienza," Rome, Italy, pers. comm.), but results are incomplete; therefore, to avoid further confusion, we have referred to our specimens as *Phocascaris* sp. There are few records of *Phocascaris* sp. from Canadian seals. Scott and Fisher (1958) did not distinguish this genus in their survey of nematodes from Canadian seals, but Myers (1957b) found *Phocascaris* in 7 of 195 harp seals collected near the Magdalen Islands in 1956. McClelland (1980a) found *Phocascaris* sp. in 7 of 53 grey seals from Nova Scotia. The present study shows that *Phocascaris* sp. will develop to maturity and is common and widely distributed in Canadian Atlantic harp seals. Our data probably underestimate the numbers of *Phocascaris* sp. in harp seals because these nematodes, particularly adults, also occur in the anterior small intestine (Palsson 1977; J. Brattey, unpubl. obs.) which was not available for examination during our study.

Although there are numerous records of *C. osculatum* in harp seals from the North Atlantic, much of the information on infection levels is anecdotal or qualitative (Berland 1963; Lyster 1940; Myers 1957a), and there are few detailed estimates of the numbers of *C. osculatum* present (Myers 1957b; Scott and Fisher 1958). Our results show that almost all harp seals off Newfoundland and Labrador harbour *C. osculatum* B and that the abundance of this nematode varies widely between individual seals, with animals typically harbouring several hundred specimens. The presence of large numbers of nematodes in the stomach of harp seals is well known among seal hunters who have assumed that these nematodes are sealworm (*P. decipiens*); apparently this has led to the misconception among sealers and fishermen that harp seals, or seals in general, are responsible for the nematode worms (larval *P. decipiens*) that occur in the flesh of commercially exploited fish species off eastern Canada (see McClelland et al. 1983a, 1983b, 1985; Brattey et al. 1990). However, our results clearly indicate that almost all the adult nematodes in harp seals from "the front" herd are *C. osculatum* B or *Phocascaris* sp. The larvae of these nematodes are primarily confined to and discarded with the viscera of marine fish and therefore do not constitute a problem for the commercial fishing industry.

The life cycles of *C. osculatum* B and *Phocascaris* sp. are poorly known (Davey 1969; McClelland and Ronald 1974) but are assumed to be similar to those of other marine ascaridoids such as *P. decipiens* (see McClelland 1990) with larval stages occurring in marine invertebrates and fish. However, the fish species involved in transmitting *C. osculatum* and *Phocascaris* sp. to harp seals are not well documented. Confusion in the literature has led to incorrect conclusions concerning the fish hosts of these nematodes. Many of the earlier records of larval *Contracaecum* sp. are probably *Hysterothylacium* sp. (= *Thynnascaris* sp. or *Contracaecum clavatum*), and the most recent synopsis of parasites of fishes of Canada gives no records of larval *C. osculatum* (Margolis and Arthur 1976). Also, according to Berland (1963) the third-stage larvae of *C. osculatum* and *Phocascaris* sp. may be morphologically indistinguishable, and most authors have not attempted to distinguish them. Nonetheless, there are reports of larval *Phocascaris* sp. in cunner, *Tautogolabrus adspersus* (Sekhar and Threlfall 1970), and larval *C. osculatum* in Atlantic cod, American plaice, *Hippoglossoides platessoides* (McClelland et al. 1985), and capelin, *Mallotus villosus* (Palsson and Beverley-Burton 1984), from Newfoundland. Capelin and Arctic cod, *Boreogadus saida*, are commonly eaten by harp seals (Sergeant 1973; Bowen 1985; Finley et al. 1990), and these species may be of major importance in the life cycles of *C. osculatum* B and *Phocascaris* sp. in Canadian waters. However, the relative importance of these and other fish and invertebrate species as hosts for *C. osculatum* B and *Phocascaris* sp. remains difficult to determine; a simple method of distinguishing the larvae of these nematodes is needed, combined with more information on the numbers of larvae infecting harp seal prey.

Most harp seal pups from the front herd are born during the middle of March (Myers and Bowen 1989) and are weaned about 12 d later (Kovacs and Lavigne 1985). They subsequently moult and undergo a fasting period during the next several weeks (Worthy and Lavigne 1983) before beginning independent feeding during mid- to late April. Our discovery of sexually mature adults of *C. osculatum* B in pups collected during the first 2 wk of May suggests, therefore, that these nematodes require approximately 2–3 wk to develop to maturity in the harp seal. Similar maturation times have been reported for *P. decipiens* in captive grey and harbour seals and a single harp seal (Scott 1953; McClelland and Ronald 1974; McClelland 1980a). Other aspects of the dynamics of *C. osculatum* B in harp seals, such as survival time and dura-

tion of the patent period, have not been determined but are fundamental to interpretation of the data reported here concerning host age and nematode infection. McClelland (1980a) found that primary infections of *P. decipiens* survived in grey seals for approximately 1–2 mo, although in later experiments, some specimens survived for as long as 5 mo (G. McClelland, pers. comm.). Since almost all harp seals are infected with *C. osculatum* B irrespective of their age (Fig. 2 and 3), an assumption of a similar life span (of up to 5 mo) for *C. osculatum* B in harp seals would suggest that there is a continuous turnover of *C. osculatum* B in the stomach of harp seals throughout their lives.

Several authors have reported higher abundances of ascaridoid nematodes among adult seals compared with juveniles and pups (Scott and Fisher 1958; McClelland 1980a), and our results agree with these findings. Stobo et al. (1990) suggested that seal size (length) rather than age is more important in determining the abundance of *P. decipiens* in grey seals. The increased abundance of *C. osculatum* B among older harp seals therefore possibly reflects the greater carrying capacity of the stomach among the larger animals. Age-related changes in quantity and size of prey items in the diet of harp seals may also result in greater numbers of larvae being ingested. More information on seal diet and the abundance of larval nematodes in harp seal prey is needed to investigate this possibility.

The frequency distributions of *C. osculatum* B among harp seals have not been described previously but appear to conform to the pattern observed in many other host–parasite systems, i.e. a negative binomial distribution with values of k less than 1. Stobo et al. (1990), however, found that the distribution of *P. decipiens* in grey seals from Sable Island was best described by the more skewed Poisson lognormal model. Changes in the shape of the dispersion pattern are of fundamental interest in understanding parasite population dynamics (Anderson 1978; Anderson and May 1978; Keymer 1982). Our data suggest that the degree of aggregation of mature adult females of *C. osculatum* B, as measured by k, tends to decline with host age. According to Pacala and Dobson (1988), this finding suggested that some form of density dependence is operating, apparently on nematode maturation in harp seals. The large proportions of larval nematodes in most stomachs are also consistent with this suggestion. Some of the other mechanisms thought to regulate host–parasite systems (see Anderson and Gordon 1982) do not appear to be involved in controlling *C. osculatum* B populations in harp seals. For example, the host age-related increase in the abundance of *C. osculatum* B, particularly of mature females, indicates that control is clearly not achieved through development of an effective long-lasting immunity to these nematodes. Also, aside from the development of gastric ulcers in some animals, heavily infected seals appeared healthy with no evidence of weight loss or emaciation. Our data for female seals suggest the contrary, with heavily infected females appearing to be in better condition; consequently, a control mechanism involving parasite-induced death of heavily infected hosts also seems unlikely.

The biased sex ratio of adult nematodes in harp seals agrees with Myers (1957b) who found an excess of females of *C. osculatum* B and *Phocascaris* in harp seals from the Gulf of St. Lawrence. In contrast, data in Young and Lowe (1969) and Young (1972) indicated a slight excess of adult male *C. osculatum* and *P. decipiens* (=*Terranova decipiens*) in grey seals from British waters. McClelland (1980a) and Stobo et al. (1990) reported sex ratios of approximately 1:1 for adult *P. decipiens* in naturally infected grey seals from Canadian waters. These comparisons indicate that there is no consistent pattern in the sex ratios among species of ascaridoid nematode infecting seals. Several factors can influence the sex ratio (Bull 1983; Tingley and Anderson 1986), and the biased sex ratio observed here may be due to adult females surviving longer than males in the stomach of seals. McClelland (1980a) found no evidence of longer survival of female compared with male *P. decipiens* in experimentally infected grey seals, but there is no information on the survival of adult male and female *C. osculatum* B and *Phocascaris* sp. The reasons for the biased sex ratios of those nematodes and for the heterogeneity of nematode sex ratios among individual harp seals require investigation.

Gastric lesions similar to those observed here have been observed in several marine mammal species, primarily in association with nematodes of the genera *Contracaecum* (Young and Lowe 1969; Wilson and Stockdale 1970; Griner 1974) and *Anisakis* (Smith 1989; Young and Lowe 1969). Aside from McClelland's (1980b) study which indicated no lesions in the stomach of 53 grey seals and 14 of 16 harbour seals infected with *P. decipiens*, there are few data on the prevalence of lesions in North Atlantic seal populations. Our results indicate that lesions associated with *C. osculatum* B and *Phocascaris* sp. are common in Canadian Atlantic harp seals and that they occur in animals of a wide range of ages and during several months of the year. The reasons for the higher prevalence of lesions in harp seals from eastern and northeastern Newfoundland compared with other areas are not known. The presence of lesions in some harp seals with few nematodes can probably be attributed to the nematodes having been recently shed.

Our discovery that female harp seals with large numbers of nematodes had a higher body condition index than lightly infected individuals was surprising and contrasts with the general biological finding that high parasite burdens are associated with poorer health. The absence of a significant association among male seals can possibly be attributed to the smaller sample size. Our results support the contention that *C. osculatum* and *Phocascaris* sp. are not particularly harmful to free-living harp seals. Experimental studies (McClelland 1980b) and evidence from autopsies (Wilson and Stockdale 1970; Keyes 1965) suggest, however, that these nematodes and related species (e.g. *Anisakis* sp.) may be more harmful to diseased or captive marine mammals that are under stress.

In conclusion, our results suggest that harp seals off Newfoundland and Labrador are not important in the transmission of *A. simplex*, but their role in the transmission of *P. decipiens* is more difficult to determine. Information on population sizes and sealworm infection levels of other pinniped species is needed before an estimate can be made of the relative importance of harp seals in the transmission of *P. decipiens*. *Contracaecum osculatum* B and *Phocascaris sp.* are clearly the most prevalent and abundant nematodes inhabiting the stomach of harp seals in the areas investigated. More information is needed on various aspects of the basic biology of these nematodes, particularly the prevalence and abundance of larvae infecting intermediate hosts, the survival time of adult male and female worms in seals, and the interactions between the various nematode species in the stomach of harp seals.

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Comparison of Three Methods of Measuring RNA and DNA Concentrations of Individual Pacific Herring, *Clupea pallasi*, Larvae

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Whole-body concentrations of RNA and DNA of individual Pacific herring, *Clupea pallasi*, larvae were measured with three methods that rely on the fluorescence of dyes bound to nucleic acids. Two methods use the dye ethidium bromide and do not purify nucleic acids, and one method uses ethidium bromide plus the DNA-specific dye bisbenzimidazole and purifies nucleic acids. Highly significant differences in RNA concentration and RNA–DNA ratios were found between the three methods. Significant differences in RNA concentration and RNA–DNA ratios were also found between yolk sac larvae and non-yolk sac larvae analyzed by one of the two ethidium bromide methods, with yolk sac larvae having the lowest ratios. These results suggest that compounds other than nucleic acids may interfere in the two ethidium bromide methods. Until a standard method for extraction and measurement of nucleic acids of larval fish is established, we recommend the use of techniques that purify nucleic acids.

Les concentrations d'ARN et d'ADN dans des larves entières de hareng du Pacifique, *Clupea pallasi*, ont été mesurées suivant trois méthodes utilisant des colorants fluorescents qui se fixent aux acides nucléiques. Deux d'entre elles utilisent le bromure d'éthidium sans qu'il y ait purification des acides nucléiques, tandis que la troisième utilise le bromure d'éthidium ainsi que le bisbenzimidazole, colorant se liant spécifiquement à l'ADN, avec purification des acides nucléiques. Les trois méthodes ont donné des concentrations d'ARN et des rapports ARN–ADN très différents. On a aussi obtenu des concentrations d'ARN et des rapports ARN–ADN différents entre des larves ayant encore leur sac vitellin et des larves où ce dernier avait été résorbé avec une des deux méthodes utilisant exclusivement le bromure d'éthidium; les larves avec sac vitellin ont donné les rapports les plus bas. Ces résultats laissent entendre que certains composés doivent interférer dans les deux méthodes utilisant exclusivement le bromure d'éthidium. Jusqu'à ce qu'une méthode normalisée d'extraction et de détermination des acides nucléiques des larves de poissons soit mise au point, nous recommandons l'utilisation de méthodes dans lesquelles les acides nucléiques sont purifiés.

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The concentration of DNA in a normal somatic fish cell is roughly constant, but the concentration of RNA varies directly with protein production. Therefore, the ratio of RNA to DNA varies directly with protein production and can be used as an index of instantaneous growth (Buckley 1984; Bulow 1987. RNA–DNA ratios are particularly useful in studies of the population dynamics of larval fishes because survival of larvae varies directly with their nutritional status. However, there is no standard technique for the measurement of fish nucleic acids. Over the last 12 yr, four different methods have been used; three were developed specifically for the very small quantities of nucleic acids in larval fish. In this paper, we investigate whether these latter three methods produce comparable results for Pacific herring, *Clupea pallasi*, larvae.

The oldest method of measuring RNA and DNA concentrations of fish larvae is the Schmidt–Thannhauser technique as described by Munro and Fleck (1966) and as adapted by Buckley (1979). It is a two-step procedure: nucleic acids are purified and then their absorbance of UV light in the 260-nm band is measured. The method has been used on a variety of larval fishes (Buckley 1979, 1980, 1981, 1982, 1984; Buckley et al. 1984; Wright and Martin 1985; Buckley and Lough 1987), including Pacific herring larvae (Fukuda et al. 1986) and Atlantic herring, *Clupea harengus* (previously *Clupea harengus harengus*), larvae (Clemmesen 1987). However, it has the disadvantage of requiring a minimum of 800 μg dry weight·sample^{-1}, which means that it cannot be used to measure the nucleic acid concentrations of individual fish larvae.

A method for measuring amounts as small as 0.05 μg DNA·mL^{-1} and 0.1 μg RNA·mL^{-1} was first introduced by LePecq and Paoletti (1966) and later modified by Karsten and Wollenberger (1972, 1977), Boer (1975), and Robinson (1988). Nucleic acids are not separated from other tissue components. Instead, the method relies on the enhanced surface fluorescence of nucleic acids when they are bound to the dye ethidium

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bromide. RNA concentration is measured by the decrease in fluorescence after the addition of the enzyme RNAse to homogenized tissue; the remaining fluorescence is assumed to be due entirely to DNA. Prasad et al. (1972) showed that this method produced results comparable with those of the Schmidt–Thannhauser method for mammalian tissue. This method has been used on Atlantic cod, *Gadus morhua*, larvae by Raae et al. (1988) and on Pacific herring larvae by Robinson and Ware (1988). It has two disadvantages: fluorescence may be absorbed or released by compounds other than nucleic acids, and DNA concentration may be overestimated if RNAse is inhibited by other compounds.

Bentle et al. (1981) proposed a modification that placed RNAse and DNAse together in one aliquot so that any residual fluorescence due to compounds other than nucleic acids or due to inhibition of RNAse is detected after incubation is complete. However, this method cannot determine the cause of residual fluorescence. The Bentle method was used by Haldorson (1989) on walleye pollock, *Theragra chalcogramma*, larvae.

In response to these concerns, Clemmesen (1988) proposed another modification; nucleic acids are purified by repeated washing of the homogenized fish tissue with organic solvents, and a DNA-specific dye, bisbenzimidazole, is used instead of ethidium bromide to measure DNA concentration. The Clemmesen method has been used on at least three species of fish larvae (Clemmesen 1988; Hovenkamp 1990), including Atlantic herring larvae (Clemmesen 1989).

Following the example of Robinson and Ware (1988), we used the Karsten–Wollenberger method as modified by Robinson (1988) on wild herring larvae collected from four sites in Prince William Sound, Alaska, in 1989. We were testing the hypothesis that the *Exxon Valdez* oil spill of March 24, 1989, reduced growth of herring larvae in the Sound. We found highly variable RNA–DNA ratios between larvae captured at the same dates from the same plankton stations. We wished to learn whether this high within-sample variability was natural or whether it was due to our application of the Karsten–Wollenberger method. To answer the question, we used the Bentle and Clemmesen methods on herring larvae taken from the same dates at one station. This paper reports the results of the comparison of the three methods.

Materials and Methods

Collection of Herring Larvae

Herring larvae were collected from a plankton station at 60°54.5′N and 146°44.9′W in Tatitlek Narrows in the northeastern part of Prince William Sound, Alaska. The slick from the *Exxon Valdez* oil spill, which had its origin 10 km to the west of the Narrows, never approached the Narrows, but drifted directly away from it towards the southwestern exit of the Sound.

The station was visited seven times: once each week between May 2 and June 22, 1989. The same sampling protocol was followed at each date. Temperature and salinity were measured at 2-m intervals from the surface to 30 m with a conductivity–temperature meter. Herring larvae were captured by towing a bongo net with a 60-cm mouth diameter, a length of 3 m, and a mesh width of 333 or 505 μm at a speed of about 1 $m \cdot s^{-1}$ in a double oblique pattern from the surface to 30 m and back. The 333-μm mesh was used for the first two cruises, when the population of larvae contained many newly hatched larvae, because herring larvae <8 mm long are extruded through 505-μm mesh (Colton et al. 1980; M. D. McGurk, unpubl. data). The 505-μm mesh was used for the remaining five cruises.

Herring larvae were picked from the fresh zooplankton with the aid of a dissecting microscope within 1–5 min after capture. Between one and three consecutive plankton tows were made to collect a maximum of about 30 larvae on each date. Each larva was placed in a small numbered plastic vial filled with seawater. The vials were immediately sandwiched between large blocks of dry ice (frozen CO_2, melting point of −56°C) and stored in an insulated chest. After the end of each 2-d cruise, the frozen larvae were flown or driven in blocks of dry ice to Anchorage where they were stored at −70°C in the freezers of the U.S. Fish and Wildlife Service. After the end of the field season the frozen larvae were flown in blocks of dry ice to the laboratory in Sidney, B.C., where they were stored at −70°C.

In the first phase of analysis, between 4 and 13 larvae were chosen randomly from the samples collected on the seven dates and analyzed with the Karsten and Wollenberger method. In the second phase, all of the remaining larvae were analyzed with either the Bentle or Clemmesen method. Larvae were chosen so that samples from at least five of the seven dates were analyzed by the latter two methods. Before processing, each larva was thawed, identified as yolked or non-yolked, and measured for standard length to the nearest 0.1 mm.

Measurement of Nucleic Acids

Karsten–Wollenberger method

Each larva was placed in 3 mL of ice-cold phosphate-buffered saline (PBS) and homogenized with a high-speed hand-held probe (Ultra Turrax) at 17 000 rpm for two 15-s treatments. Two 0.5-mL aliquots were used to measure total nucleic acids, which meant adding heparin to dissociate the nucleoproteins plus ethidium bromide and then measuring total fluorescence. Two more aliquots were used to measure DNA concentration by adding 0.5 mL of heparin plus 0.5 mL of RNAse (purified from bovine pancreas; Sigma No. R-4875) to digest the RNA, leaving only DNA. A fifth aliquot was used to measure background fluorescence; no ethidium bromide was added to that aliquot. A sixth blank aliquot containing only PBS was run each day to calibrate the fluorometer. Robinson (1988) modified this method by increasing the time that the DNA aliquots were incubated with RNAse from 20 to 30 min and by increasing the concentration of RNAse from 50 to 100 $\mu g \cdot mL^{-1}$.

The fluorometric response to known concentrations of nucleic acids was measured in triplicate at eight concentrations of DNA (purified from herring testes; Sigma No. D-6898): 0.05, 0.1, 0.25, 0.5, 1, 2, 3, and 4 $\mu g \cdot mL^{-1}$, and six concentrations of RNA (purified from calf liver; Sigma No. R-7250): 0.25, 1, 3, 5, 10, and 15 $\mu g \cdot mL^{-1}$. Four independent tests showed that the RNA and DNA standards were not contaminated with each other, so no adjustments to the standard curves were necessary.

Bentle method

A different ionic medium, buffer C (20 mM Tris–HCl at a pH of 7.5, 1 mM MgCl, 0.8 mM CaCl, and 50 mM NaCl), was used instead of PBS to optimize the activity of DNAse when it was used in combination with RNAse. Each larva was homogenized in 3 mL of ice-cold buffer C. Ethidium bromide was added to two aliquots and the mean concentration of total

nucleic acids was measured fluorometrically. Then, RNAse to a final concentration of 25 $\mu g \cdot mL^{-1}$ was added to two other aliquots in order to digest all RNA and give a concentration of DNA. Finally, RNAse plus DNAse I (purified from bovine pancreas; Sigma No. D-5025) to a final concentration of 5 $\mu g \cdot mL^{-1}$ was added to another two aliquots in order to digest all nucleic acids and give a residual fluorescence. Volumes of aliquots and incubation times were the same as in the Karsten–Wollenberger method. A separate set of standard curves was prepared.

Clemmesen method

Each larva was homogenized in an ice-cold buffer containing 0.3 mL of 0.05 M Tris–HCl, 0.1 M NaCl, 0.01 M EDTA at a pH of 9.0, and 0.2 mg proteinase $K \cdot mL^{-1}$. Sodium dodecyl sulfate (SDS), at a final concentration of 2%, was then added. The homogenate was centrifuged at 6000 rpm for 15 min and then the supernatant was decanted into a new vial into which 0.3 mL of 80% phenol and 0.3 mL of chloroform–isoamylalcohol (24:1) were added. The solution was mixed for 10 min and then centrifuged at 6000 rpm for 10 min. The phenol–chloroform–isoamylalcohol phase was discarded and the aqueous phase containing the nucleic acids was washed a second time. The aqueous phase from that washing was extracted, combined with 0.3 mL of the chloroform–isoamylalcohol mixture, mixed for 1 min, and then centrifuged for 5 min. This procedure was repeated a second time in order to completely wash the aqueous phase. Finally, 0.2 mL of buffer was added to the aqueous phase and it was separated into several aliquots. Ethidium bromide was added to one aliquot and total nucleic acid concentration was measured from its fluorescence. Bisbenzimidazole was added to another aliquot and DNA concentration was measured fluorometrically. RNA concentration was calculated as the difference between total nucleic acid and DNA concentration. There was sufficient material to allow two measurements of total nucleic acid concentration and DNA concentration for each fish. A separate standard curve for DNA was made, and the fluorometer was blanked every day.

Statistical Analysis

RNA and DNA concentrations were expressed as micrograms per fish. Differences between methods in RNA and DNA concentrations and RNA–DNA ratios were assessed with a mixed-model analysis of variance (ANOVA):

$$Y_{ijkl} = \mu + A_i + \beta_j + C_k + (AC)_{ik} + e_{ijkl}$$

where Y_{ijkl} = the response of the lth larva of the kth size class captured on the jth day of the year and analyzed with the ith method, μ = grand mean, A_i = random effect of method i, β_j = fixed effect of day of year j, C_k = random effect of larval size class k, $(AC)_{ik}$ = random effect of the interaction of method i and size class k, and e_{ijkl} = error term. Day of year is a fixed effect because we assume that there were no relationships between environmental conditions on a sampling day and the response of a biochemical method performed in the laboratory several months later. Since there can be no interactions between fixed and random variables, there are no interactions between day of year and method or between day of year and size class (Sokal and Rohlf 1981).

In all statistical tests, RNA and DNA concentrations and the RNA–DNA ratio were ln-transformed to normalize their distributions. Larvae were grouped into three size classes: yolked, non-yolked between 6.0 and 11.9 mm long, and non-yolked between 12.0 and 23.9 mm long. Date was expressed as day of year (DOY), previously known in the fisheries literature as Julian date. It was grouped into two classes: May 2–20 (DOY = 122–140) and May 30 – June 20 (DOY = 150–171).

Multiple regression was used to describe variation in ln(RNA), ln(DNA), and ln(RNA–DNA) with method, larval length, presence–absence of yolk, and day of year. Dummy variables were used for the three methods and for the presence–absence of yolk. Only the factors that were identified by ANOVA as statistically significant at the 1% probability level were entered into the regression. All r^2 were adjusted for the number of degrees of freedom in the regression model.

Estimated Protein Growth and Duration of Starvation

Instantaneous rates of protein growth, G_{pi} (percent per day), of herring larvae were estimated from RNA–DNA ratios and mean temperature at date of capture, T (degrees Celsius), with Buckley's (1984) equation (3):

$$G_{pi} = 0.93T + 4.75(\text{RNA–DNA ratio}) - 18.18.$$

This equation was based on an RNA–DNA range of 1.92–4.90 and a G_{pi} range of 2.2–19.5%·d^{-1}.

Duration of starvation was estimated from RNA–DNA ratio and live dry weight, W (micrograms), with Clemmesen's (1987) equation (3):

$$\text{Degree-days} = 66.373 - 27.638\log_{10}(W) - 53.387\,(\text{RNA–DNA})$$

and then converted to the number of days of starvation by dividing degree-days by mean temperature at date of capture. This equation was based on an RNA–DNA range of 0.9–3.5 and a range of durations of starvation of 0–11 d. Formalin-fixed dry weight was estimated from larval length with a weight–length regression for wild herring larvae from Prince William Sound (McGurk et al. 1990); it was increased by 10% to account for losses in live dry weight upon fixation and storage in 3% formalin.

Estimates of G_{pi} and duration of starvation were not subjected to statistical analysis because many were extrapolations; the range of RNA–DNA ratios measured in this study exceeded the ranges used to develop Buckley's (1984) and Clemmesen's (1987) equations. Instead, we examined trends in G_{pi} and duration of starvation between methods and used these data to support conclusions derived from statistical analysis of RNA–DNA ratio.

Results

Sampling

Sampling began on May 2, 1989, soon after the first cohort of herring larvae hatched into Tatitlek Narrows from a nearby beach. This was shown by the fraction of larvae that carried a yolk; 26 of the 27 (96%) larvae captured on May 2 were yolked, but this fell to 6 of the 30 (20%) larvae captured on May 11 and to 0% on all subsequent dates. Sampling ended on June 22, 1989, when the density of larvae was so low that only four larvae were captured after three consecutive plankton tows. Over that time period the mean temperature of the upper 30 m rose from 5.1 to 8.3°C at an average rate of 0.08°C·d^{-1} (Fig. 1).

Most larvae were either dead or dying when the codend of the net was opened; many exhibited the paleness that indicates osmotic shock caused by tumbling in the codend of the net.

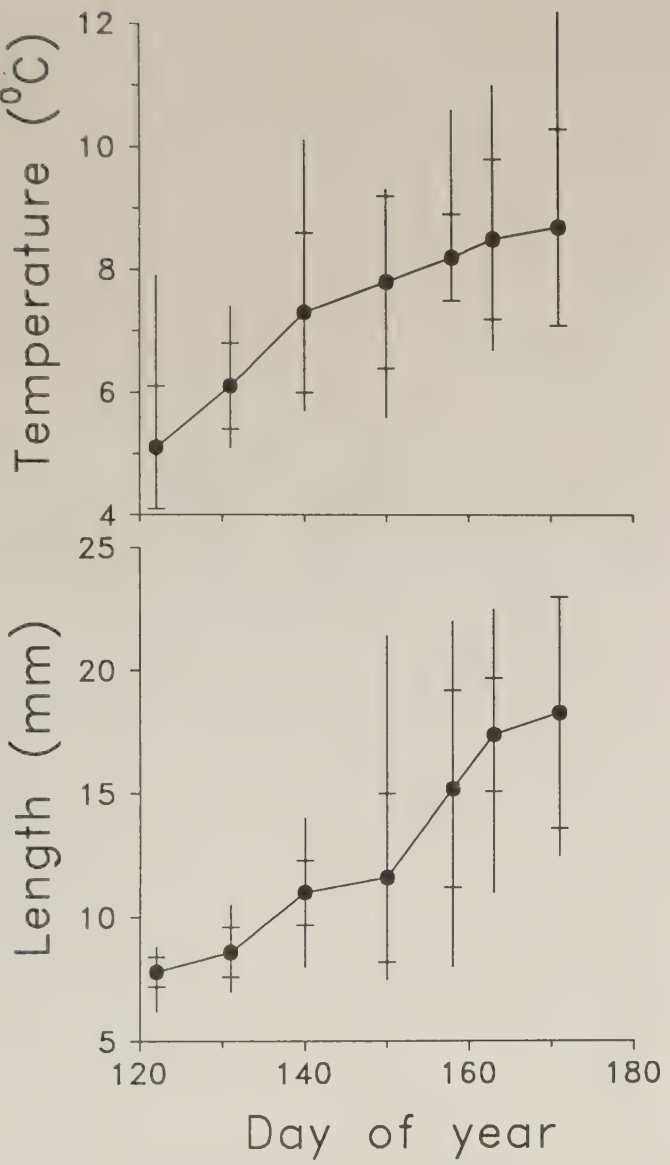

FIG. 1. Water temperature of the upper 30 m of a plankton station in Tatitlek Narrows, Prince William Sound, and growth in length of herring larvae captured at that station. Closed circles are means, horizontal bars are ± 1 SD of the mean, and vertical bars are ranges. Sample size of each mean temperature was 16, and sample size of each mean length is shown in Table 1.

A total of 159 larvae were analyzed: 71 larvae with the Karsten–Wollenberger method, 30 with the Bentle method, and 58 with the Clemmesen method (Table 1).

Growth in Length

Larvae ranged in length from 6.2 to 23.0 mm. There were no significant ($P > 0.05$) differences in mean larval length-at-date between the three methods, but there was a highly significant ($P < 0.001$) increase in mean length with day of year. Larvae grew at an average rate of 0.23 mm·d^{-1} over the sampling period (Fig. 1). A sudden increase in the variance of mean length after May 30 (DOY = 150) was due to the movement of a second cohort of herring larvae into Tatitlek Narrows on or before that date (McGurk et al. 1990). These larvae did not carry a yolk sac, which suggests that they did not hatch from the same beaches as the first cohort.

TABLE 1. Number of herring larvae and mean (± 1 SD) length, RNA and DNA concentrations, and RNA–DNA ratios analyzed with three methods: K = Karsten–Wollenberger method, B = Bentle method, C = Clemmesen method.

	May				June		
	2	11	20	30	7	15	20
				Number of larvae			
K	10	10	10	13	9	15	4
B	—	9	8	8	—	5	—
C	17	11	10	10	—	10	—
				Length (mm)			
K	8.0 (0.3)	8.6 (1.1)	11.2 (1.7)	12.4 (4.1)	15.2 (4.0)	17.0 (2.5)	18.3 (4.7)
B	— —	8.8 (1.0)	11.6 (0.9)	10.8 (2.8)	— —	18.9 (2.0)	— —
C	7.6 (0.6)	8.5 (1.0)	10.4 (1.1)	11.1 (3.0)	— —	17.1 (2.1)	— —
				DNA (μg·fish^{-1})			
K	0.835 (0.190)	0.819 (0.261)	2.255 (0.395)	1.910 (1.132)	2.287 (1.463)	3.230 (1.162)	7.318 (6.551)
B	— —	0.883 (0.294)	1.528 (0.521)	1.796 (0.932)	— —	4.543 (1.535)	— —
C	0.536 (0.181)	0.788 (0.240)	1.582 (0.356)	1.485 (0.444)	— —	3.199 (1.474)	— —
				RNA (μg·fish^{-1})			
K	1.077 (1.018)	2.066 (0.707)	4.888 (2.665)	13.264 (9.192)	12.172 (12.035)	18.937 (9.781)	70.644 (72.985)
B	— —	3.504 (0.992)	7.284 (2.936)	7.367 (5.853)	— —	16.433 (11.083)	— —
C	3.838 (1.339)	5.120 (1.524)	10.411 (2.811)	9.674 (5.405)	— —	22.653 (7.153)	— —
				RNA–DNA ratio			
K	1.345 (1.255)	2.935 (2.200)	2.102 (0.875)	7.603 (3.154)	5.868 (4.865)	5.730 (1.941)	8.192 (2.848)
B	— —	4.572 (2.500)	5.511 (3.386)	3.868 (1.613)	— —	3.386 (1.006)	— —
C	8.142 (4.414)	6.802 (3.577)	7.043 (3.184)	6.365 (2.642)	— —	7.555 (1.916)	— —

Absorbance of Fluorescence by Yolk

The mean RNA concentration and mean RNA–DNA ratio of yolk sac larvae were both significantly ($P < 0.001$) lower for the Karsten–Wollenberger method than for the Clemmesen method (Fig. 2). Meaningful comparisons with the Bentle method were not possible because there was only one yolk sac larvae in that group. This finding suggests that components of yolk may have absorbed RNA fluorescence in the Karsten–Wollenberger method. This finding justified placing yolked larvae into their own size class in ANOVA.

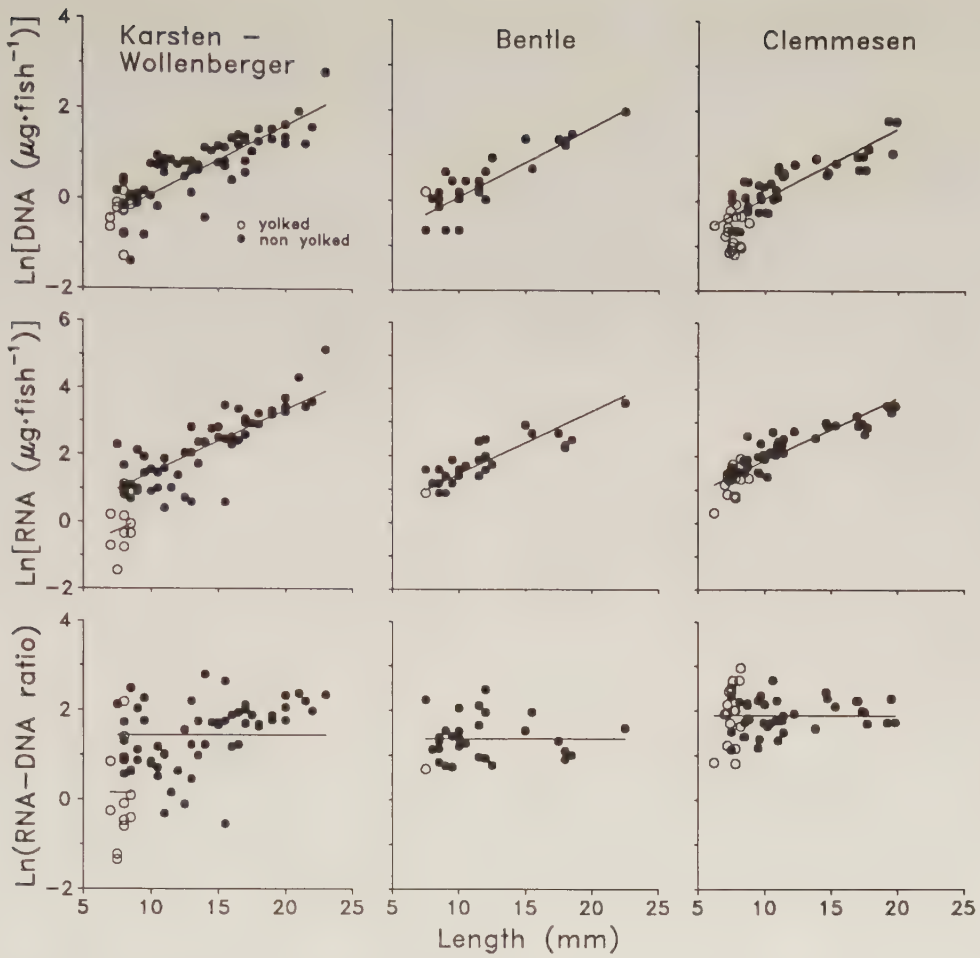

FIG. 2. Plots of ln(RNA), ln(DNA), and ln(RNA–DNA) of herring larvae against larval length for three methods of measurement. Lines are concentrations or ratios predicted by the regression equations of Table 3.

TABLE 2. ANOVA of ln(DNA), ln(RNA), and ln(RNA–DNA) of herring larvae. Sample size was 159.

Source of variance	df	ln(DNA)		ln(RNA)		ln(RNA–DNA)	
		F-ratio	*P*	*F*-ratio	*P*	*F*-ratio	*P*
Within cell	145						
Constant	1	23.80	<0.001	406.66	<0.001	300.54	<0.001
Method	2	2.31	0.103	25.35	<0.001	39.11	<0.001
Larval size	2	38.55	<0.001	33.67	<0.001	2.65	0.074
Day of year	1	1.00	0.319	5.64	0.019	2.94	0.089
Method·size	4	1.39	0.240	4.42	0.002	7.40	<0.001

Nucleic Acid Concentrations

Ln(DNA) increased linearly with larval length, but did not vary significantly with method, day of year, or the interaction of method and larval size (Tables 2 and 3; Fig. 2). Ln(RNA) also increased linearly with larval length, but there were highly significant differences in ln(RNA)-at-length between methods and between yolked and non-yolked larvae. Regression showed that the Clemmesen method estimated significantly higher ln(RNA)-at-length than the other two methods and that the Karsten–Wollenberger method applied to yolk sac larvae estimated the lowest values of RNA. Ln(RNA)-at-length measured by the Bentle method was indistinguishable from that of non-yolked larvae measured by the Karsten–Wollenberger method.

Ln(RNA–DNA) did not vary with length for all non-yolked larvae, but mean ln(RNA–DNA) was significantly different between methods and between yolked and non-yolked larvae. The regression equation showed that the method–yolk categories were, in order of decreasing geometric mean RNA–DNA ratio, as follows: Clemmesen method, Karsten–Wollenberger method for non-yolked larvae, Bentle method, and Karsten–Wollenberger method for yolked larvae.

Protein Growth Rates

There was a striking difference in mean G_{pi} of yolk sac larvae between the Karsten–Wollenberger method, $-3.93\%\cdot d^{-1}$, and the Clemmesen method, $25.98\%\cdot d^{-1}$ (Fig. 3). Since it is not possible for yolk sac larvae to starve because they have an endogenous food supply, Buckley's (1984) model implies that the RNA–DNA ratios of most yolk sac larvae measured by the Karsten–Wollenberger method were underestimated.

TABLE 3. Regressions of ln(DNA), ln(RNA), and ln(RNA–DNA ratio) on length of herring larvae and on dummy variables for three methods of biochemical analysis and the presence–absence of yolk. Sample size was 159. SE is the standard error of the regression coefficients.

	Coefficient	SE	P	r^2
ln(DNA)				
Intercept	−1.478	0.400	<0.001	0.725
Length	0.1549	0.0676	<0.001	
ln(RNA)				
Intercept	−0.4316	0.1470	0.004	0.777
Length	0.1882	0.0104	<0.001	
Clemmesen	0.4246	0.0891	<0.001	
Yolked·Karsten–Wollenberger	−1.236	0.1589	<0.001	
ln(RNA–DNA ratio)				
Intercept	1.902	0.0835	<0.001	0.337
Karsten–Wollenberger	−0.4605	0.1181	<0.001	
Bentle	−0.5317	0.1430	<0.001	
Yolked·Karsten–Wollenberger	−1.291	0.1951	<0.001	

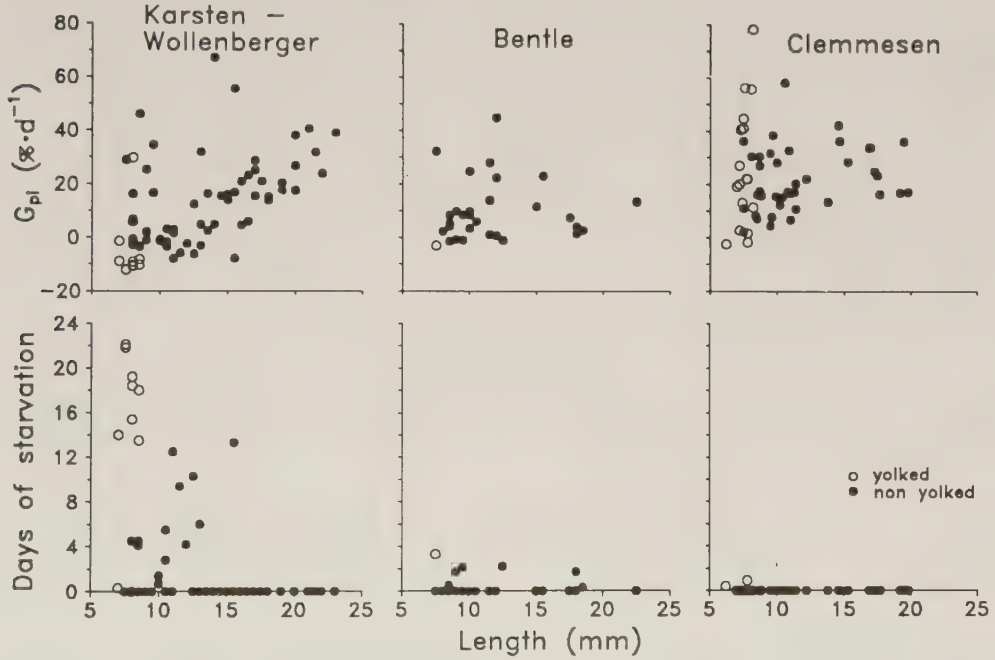

FIG. 3. Plots of estimated protein growth rate and number of days of starvation of herring larvae against length for three methods of measuring nucleic acids.

There was a large difference between methods in the fraction of larvae classified as starving, i.e. $G_{pi} < 0\%\cdot d^{-1}$. Thirty-seven percent of larvae measured by the Karsten–Wollenberger method were classified as starving, compared with 17% measured by the Bentle method and 3% by the Clemmesen method.

Duration of Starvation

Yolk sac larvae analyzed by the Karsten–Wollenberger method were classified by Clemmesen's (1987) equation as starving for more than 10 d before capture. In contrast, the duration of starvation for yolk sac herring larvae measured by the Bentle and Clemmesen methods was estiamted to be <4 d. At temperatures ranging from 5 to 8°C, yolk would have been exhausted in only 7–10 d (Alderdice and Velsen 1971; McGurk et al. 1990), which implies that the Karsten–Wollenberger method must have underestimated RNA–DNA ratios.

Discussion

This study shows that RNA–DNA ratios of individual herring larvae vary with the method of measurement. In the absence of any independent measure of RNA–DNA ratio, we have no means of determining which method is most accurate. However, it is reasonable to assume that the Clemmesen method is less likely to be biased than the other two methods, simply because it purifies nucleic acids before measuring them. This study provides three lines of evidence to support this assumption.

First, the Clemmesen method had significantly higher RNA–DNA ratios than the Karsten–Wollenberger and Bentle methods, which suggests that compounds other than nucleic acids may have interfered in the measurement of fluorescence, perhaps by absorbing fluorescence or by contributing to residual fluorescence or by inhibiting the kinetics of RNAse and DNAse. The data of this study do not indicate which mechanism is most likely. The key to this argument is not the ranking of mean RNA–DNA ratios because the geometric mean RNA–DNA ratios for the Karsten–Wollenberger and Bentle methods are

not unusually low; they fall within the range of mean RNA–DNA ratios-at-length reported for Pacific herring larvae by Fukuda et al. (1986, fig. 3) and Robinson and Ware (1988, table 2) and they are greater than the RNA–DNA ratios-at-age reported for Atlantic herring larvae by Clemmesen (1987, fig. 1). Instead, the argument depends on the correlation between high ratios and purification of nucleic acids.

Second, lower RNA–DNA ratios in yolk sac larvae than in non-yolked larvae that were analyzed by the Karsten–Wollenberger method support a link between the presence of yolk and variation in measurement of nucleic acids. Robinson and Ware (1988, fig. 2) also reported lower RNA–DNA ratios for yolk sac larvae than for non-yolk sac larvae sampled 4 d later from the same population.

Third, published regression models that relate RNA–DNA ratios to protein growth rates and to the duration of starvation show that the Karsten–Wollenberger method may seriously underestimate RNA–DNA ratios, particularly those of yolk sac larvae.

Based on these arguments, we conclude that the high within-sample variability we observed in herring larvae from Prince William Sound was more likely due to the Karsten–Wollenberger method than to natural phenomena.

If correct, these arguments imply that the Clemmesen method may also be more precise than the other two methods because the concentrations of compounds other than nucleic acids will vary between herring larvae depending on stage of development or nutritional status. This will introduce variability into the Karsten–Wollenberger and Bentle methods that will not appear with the Clemmesen method. This conclusion is supported by the observation that the variance of mean RNA–DNA ratios that we measured with the Karsten–Wollenberger method was higher than those of the other two methods. Other workers have also observed high variability and low reproducibility of the ethidium bromide methods (Richard et al. 1991; C. Clemmesen, Institut für Meereskunde an der Universität Kiel, Düsternbrooker Weg 20, 2300 Kiel, Germany, pers. comm.).

The variance of RNA–DNA ratios that we measured with the Karsten–Wollenberger method was higher than that reported by Robinson and Ware (1988) for herring larvae of southern British Columbia. We suggest that the reasons for this difference do not lie in methods of sampling or storage of larvae, or in the application of the Karsten–Wollenberg method, because the methods we used were identical to those used by Robinson and Ware (1988). The exception was the use of dry ice instead of liquid nitrogen for temporary (2–3 d) storage of herring larvae at sea. However, this could not have affected the quality of the samples because the high RNA–DNA ratios measured by the Clemmesen method were obtained from larvae stored under the same conditions. In regard to this matter, we note that Buckley and Bulow (1987) recommend that short-term storage of fish larvae at or below -15°C is adequate. Instead, we suggest that the differences were caused by conditions that were not bounded by the experimental protocol.

This is the first study to compare methods of measuring nucleic acids in individual fish larvae. Our conclusions require verification because we tested only one type of tissue and because of the nature of this study's experimental design. For logistical rasons, we were obliged to use one biochemical method on each fish and employ ANOVA techniques to control for ancillary variables such as larval size and date of capture. All other factors being equal, it would have been preferable to apply all three methods to replicate pools of larval fish. In fact, preliminary analysis of such an experimental design, using yolk sac herring larvae, found results similar to those reported here (M. D. McGurk and W. C. Kusser, unpubl. data). However, even though such experimental designs are necessary to calibrate methods, they would not reveal the biochemical mechanisms that cause differences between methods.

Until a standard method for the extraction and quantification of nucleic acids from individual fish larvae is established, we recommend that all RNA–DNA measurements be performed on purified nucleic acids.

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Temporal Variance Function for Total Phosphorus Concentration

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France, R. L., and R. H. Peters. 1992. Temporal variance function for total phosphorus concentration. Can. J. Fish. Aquat. Sci. 49: 975–977.

General relationships between means and variances can be used to determine requisite sample number for desired levels of precision but have not been developed for phosphorus, one of the best indicators of lake eutrophication. Data from 65 north-temperate lake-years are used to compare such relationships of temporal variance as functions of mean concentration for both total phosphorus (TP) and chlorophyll *a* (Chl *a*). We found TP to be less seasonally variable than Chl a, confirming several regional analyses and strengthening the established recommendations that variability in Chl a should dictate sampling program design.

Les relations générales entre les moyennes et les variances peuvent être utilisées pour déterminer le nombre d'échantillons requis afin d'obtenir le niveau de précision visé, mais elles n'ont pas été établies dans le cas du phosphore, un des meilleurs indicateurs de l'eutrophisation lacustre. Les auteurs ont utilisé 65 séries de données sur des lacs boréaux tempérés pour comparer de telles relations de variance temporelle à titre de fonctions de la concentration moyenne de phosphore total (PT) et de chlorophylle a (Chl *a*). Ils ont ainsi établi que la concentration de PT variait moins que celle de Chl a en saison. Ceci étaye les résultats de plusieurs analyses régionales et renforce les recommandations formulées à l'effect que la variabilité de la concentration de Chl *a* devrait régir la conception d'un programme d'échantillonnage.

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Determining the uncertainty associated with limnological variables is essential to the scientific treatment of seasonality (e.g. Marshall and Peters 1989), trophic interactions (e.g. Carpenter and Kitchell 1987), and sampling design (e.g. Ryding 1981; Thorton et al. 1982; Heyman et al. 1984). This consideration is needed because single measurements of total phosphorus (TP) and chlorophyll *a* (Chl *a*) concentration can differ so greatly from the mean annual values (Prepas 1979; Marshall et al. 1988) that intraannual variability often exceeds that among sites or years (El-Shaarawi and Kwiatkowski 1977; Knowlton et al. 1984; Hanna and Peters 1991). Measurements of TP and Chl *a* are used for water quality assessment (e.g. Dillon and Rigler 1974), interpreting trophic theory (e.g. McQueen et al. 1986), and predicting lake productivity (e.g. Peters 1986), yet the effect of sampling designs on the accuracy of these estimates has been considered only rarely.

One descriptor of data variability examines the linear relationship between the logarithmically transformed means and variances (Taylor 1961) to predict the requisite sample number as a function of desired precision for particular mean values (e.g. France 1988). Such variance functions can be approached on either regional (Knowlton et al. 1984; Walmsley 1984) or global (Marshall et al. 1988) scales. Regional studies usually involve preliminary sampling and therefore more accurately reflect conditions in areas of particular interest. Because the cost of sampling such sites may be considerable, many forego that step and instead rely upon general variance functions as the necessary alternative.

Marshall et al. (1988) determined the requisite sample number for water quality surveys on the basis of Chl *a*. They recommended that because "chlorophyll-*a* usually varies more than total phosphorus," Chl *a* variability should therefore dictate sampling protocol. Empirical evidence to support this contention is based on data sets of restricted geographic breadth: 2 yr for Lake Ontario (Kwiatkowski and El-Shaarawi 1977; Kwiatkowski 1985), 25 Swedish lakes (Ryding 1981; Heyman et al. 1984), and 189 lakes and reservoirs located in Missouri, Iowa, and Minnesota (Knowlton et al. 1984). In contrast, an analysis of TP and Chl *a* variation in 16 lakes in Quebec (Hanna and Peters 1991) suggested that TP was more variable seasonally than Chl *a*. The purpose of the present study was to compare the contemporaneous seasonal variation of TP and Chl *a* from a broad and independent set of north-temperate lakes to test Marshall et al's (1988) contention.

Methods

Time series showing intraannual variability in concentration of TP and Chl *a* were obtained for 65 north-temperate lake-years (Megard 1972; Schindler and Fee 1974; Dillon 1975; Gelin 1975; El-Shaarawi and Kwiatkowski 1977; Gelin and Ripil 1978; MOE 1973, 1982; Lean et al. 1983; Shapiro and Wright 1984; Kwiatkowski 1982, 1985; Rask et al. 1985; Gibson et al. 1988; Barica 1975, 1990; D. McQueen, York University, Toronto, Ont., unpubl. data).

Marshall et al. (1988) found that bias in estimation of Chl *a* occurs when fewer than five samples are collected during the year. Using the published data, we therefore determined the TP and Chl *a* values for each lake a total of seven times at mid-monthly intervals from April through October. This protocol ensured that a standardized sampling program of equal replication and interval was applied to all study lakes. Data presented in figures were read with a HIPADc digitizing pad (Houston Instruments Austin, TX).

General variance functions (Taylor 1961) for both TP and Chl *a* were calculated from the regressions of the logarithmi-

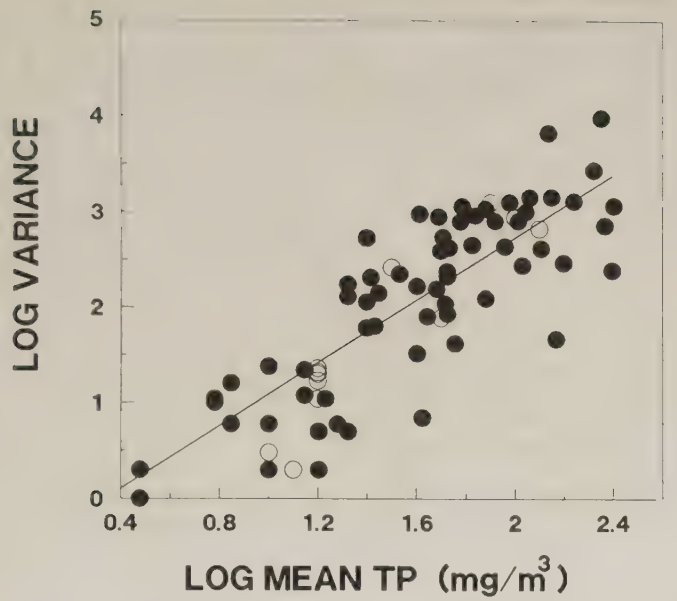

FIG. 1. Relationship between mean TP and temporal variance for 65 north-temperate lake-years from the literature. Open circles denote data from Marshall et al. (1988) not used in regression analysis.

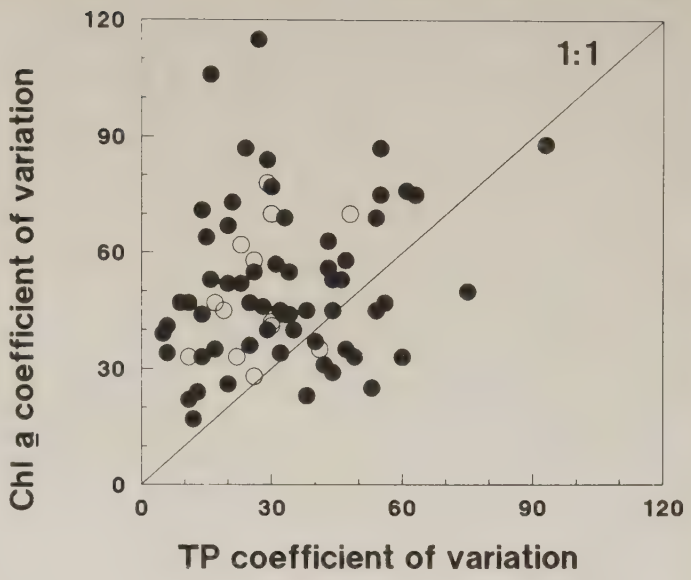

FIG. 2. Relationship between lake-specific relative variability (i.e. coefficient of variation) for contemporaneous Chl *a* and TP seasonal data. Open circles are data from Marshall et al. (1988).

cally transformed values of means ($\bar{X}$) and variances (s^2). The magnitude of relative variation between the two parameters was compared by plotting the lake-specific coefficients of variation. For 13 of the lake-years examined by Marshall et al. (1988), contemporaneous TP and Chl *a* measurements existed. These additional data were also plotted to evaluate the generality of conclusions raised by the present analysis about whether TP or Chl *a* is the more variable water quality measure.

Results and Discussion

The regression to describe temporal variance of mean Chl *a* from the present study is

$$\log s^2 = -0.61 + 2.00\,(\log \bar{X});\ R^2 = 0.90,\ S_{xy} = 0.32.$$

This relationship is very similar to those derived by Walmsley (1984) for South African lakes, Walker (1985) for Vermont lakes, and Marshall et al. (1988) for different north-temperate lakes and reservoirs.

Likewise, the temporal variance function for TP is

$$\log s^2 = -0.56 + 1.65\,(\log \bar{X});\ R^2 = 0.68,\ S_{xy} = 0.54.$$

Data from Marshall et al. (1988) follow the same pattern as those from the present analysis (Fig. 1).

Comparison of the two variance functions reveals four salient features:

(1) Chl *a* displays greater temporal variability about the seasonal mean than does TP. Because 97% or more of the values in Equations 1 and 2 are greater than the two intercepts, which in turn are approximately equal, the fact that the slope or "index of aggregation" (Taylor 1961) for the Chl *a* variance function is steeper indicates that a larger number of replicate samples is needed to achieve the same level of precision as that for TP. For any given lake, the variance for Chl *a* will actually be smaller than for TP, simply because its absolute values are generally less. However, the lake-specific values of relative variability (i.e. the coefficients of variation) of Chl *a* are greater than those of TP for 82% of the cases examined (Fig. 2). This is important because sampling program criteria are based on relative not absolute variances.

(2) The variance function for TP is not as precise as that for Chl *a*. This indicates that those factors which determine mean Chl *a* concentration also closely regulate its seasonal pattern of variation. In contrast, those factors which influence seasonal TP variability may do so irrespective of the overall lake trophic status.

(3) Although the intercepts are similar, the different slopes of the variance functions indicate that the seasonal interrelationships between TP and Chl *a* diverge with increasing lake trophic status. Such an uncoupling of the phosphorus regulation of algal biomass is probably due to the greater role of other factors such as alternative nutrient dependence, bacterial interference, and photoinhibition, all of which develop in more eutrophic systems (Prairie et al. 1989; Currie 1990; Stauffer 1991).

(4) Based on the slopes, the most successful transformations for stabilizing variance (France 1987) should be logarithmic for Chl *a* and the fourth root for TP. The importance of the more precise fourth root, over the customary logarithmic transformation in practical terms for parametric analyses using TP, remains to be established, however.

The present examination of intraannual variability among lakes on a broad geographic scale supports several previous regional analyses which indicate that Chl *a* is more seasonally variable than TP. Sampling programs designed to develop nutrient – Chl *a* relationships among lakes should therefore be based upon recommendations of five to seven temporal replicates established with respect to Chl *a* (Marshall et al. 1988).

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Relationship between Concentrations of Copper and Zinc in Water, Sediment, Benthic Invertebrates, and Tissues of White Sucker (*Catostomus commersoni*) at Metal-Contaminated Sites

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Miller, P. A., K. R. Munkittrick, and D. G. Dixon. 1992. Relationship between concentrations of copper and zinc in water, sediment, benthic invertebrates, and tissues of white sucker (*Catostomus commersoni*) at metal-contaminated sites. Can. J. Fish. Aquat. Sci. 49: 978–984.

The relationship of concentrations of copper (Cu) and zinc (Zn) in white sucker (*Catostomus commersoni*) tissues to concentrations of those metals in water, sediment, and benthic invertebrates (food) were investigated in a field study. Fish were collected from six northern Ontario lakes contaminated with mixed-metal mining wastes. The concentrations of Cu in invertebrates were correlated with water but not with sediment Cu concentrations. Conversely, Zn concentrations in invertebrates were correlated with sediment but not with water Zn concentrations. There were differences among fish from different lakes in the concentrations of Cu and Zn in liver, kidney, gill, and bone (Zn only). There were no significant correlations between tissue metal and invertebrate metal concentrations. This study suggests that liver and kidney are better indicators of chronic Cu and Zn exposure than muscle. Elevated Zn concentrations were reflected in bone tissue. For both metals, the water concentration was a better predictor of fish tissue contamination than the concentrations in either sediment or invertebrates.

La relation existant entre les concentrations de cuivre (Cu) et de zinc (Zn) dans les tissus du meunier noir (*Catostomus commersoni*) et les concentrations de ces métaux dans l'eau, les sédiments et des invertébrés benthiques dont se nourrit cette espèce a été étudiée dans le cadre d'une étude menée sur le terrain. Des poissons ont été capturés dans six lacs du nord de l'Ontario contaminés par des déchets miniers contenant divers métaux. Les concentrations de Cu dans les invertébrés étaient corrélées avec les concentrations de Cu dans l'eau, mais pas avec les concentrations de Cu dans les sédiments. Inversement, les concentrations de Zn dans les invertébrés étaient corrélées avec les concentrations de Zn dans les sédiments, mais pas avec les concentrations de Zn dans l'eau. Les concentrations de Cu et de Zn dans le foie, les reins, les branchies et les os (Zn seulement) différaient selon les lacs. On n'a pas observé de corrélations significatives entre les concentrations de ces métaux dans les tissus des poissons et leurs concentrations dans les invertébrés. Cette étude laisse entendre que le foie et les reins sont de meilleurs indicateurs de l'exposition chronique au Cu et au Zn que le tissu musculaire. Le tissu osseux reflétait bien les fortes concentrations de Zn. La concentration des deux métaux dans l'eau était un meilleur prédicteur de la contamination des tissus des poissons que leurs concentrations dans les sédiments ou les invertébrés.

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Water quality objectives use waterborne metal concentrations as indicators of impacts on aquatic life. Sediments, however, represent the most concentrated pool of metals in aquatic environments, metals that can be assimilated by the aquatic organisms resident in those sediments (Luoma 1983). Since these organisms are the primary food source for bottom-feeding fish, the accumulation of metals by such fish potentially depends on uptake from food as well as from water.

The relative importance of these two routes of metal uptake remains unclear (Luoma 1983; Dallinger et al. 1987). Several authors (Hodson et al. 1978; Hughes and Flos 1978; Part and Svanberg 1981; Thomas et al. 1983; Dallinger et al. 1987) have demonstrated that gills play an important, perhaps even a dominant, role in metal uptake. For nutritionally essential metals such as copper (Cu) and zinc (Zn), however, uptake through the gastrointestinal system of fish may also be important (Dallinger and Kautzky 1985). Several studies suggest that food and particulates are a much more important source of metals than water. Hoss (1964) found that Zn accumulation from food was more important than from water in the flounder (*Paralichthys* sp.). Pentreath (1973a, 1973b) found that water was unimportant in the uptake of Zn, manganese (Mn), cobalt (Co), and iron (Fe) in plaice (*Pleuronectes platessa*).

Since most studies on the relative importance of the dietary route for nutritionally essential metals have determined uptake from manufctured diets in a laboratory setting, they are not directly applicable to free-living fish. In the natural environment, numerous modifying factors may interact to alter the relative importance of the dietary and waterborne routes. This work was undertaken to determine the best predictor (water, sediment, or benthic invertebrate Cu and Zn concentrations) of Cu and Zn levels in the tissues of free-living white sucker (*Catostomus commersoni*) from contaminated environments.

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We also attempted to determine which white sucker tissue is the optimal indicator of chronic exposure to Cu and Zn.

Materials and Methods

The Manitouwadge chain consists of a number of small lakes (80–320 ha) near the headwaters of the Black River in northern Ontario (approximately 49°N, 85°45′W) where Cu, Zn, and silver (Ag) have been actively mined since 1957 (Fig. 1). Metal-contaminated runoff reaches the chain through both Manitouwadge and Mose lakes. The lakes are of intermediate hardness (108–112 mg·L^{-1} as $CaCO_3$) and neutral pH (6.9–7.1) (Munkittrick and Dixon 1988) and have historically shown elevated levels of Cu and Zn in water (13–30 μg Cu·L^{-1}; 180–540 μg Zn·L^{-1}) and sediment (156–2313 mg Cu·kg^{-1}; 675–7080 mg Zn·kg^{-1}) (German 1971).

The waterborne Cu and Zn concentrations in the contaminated lakes (Table 1) are currently in excess of Canadian water quality objectives (3 μg Cu·L^{-1}; 30 μg Zn·L^{-1}) (CCREM 1987). The concentrations of these metals in the sediments of the contaminated lakes are three to four orders of magnitude higher than in the water. There are currently no Canadian objectives for Cu and Zn concentrations in sediments.

Eight lakes from the Manitouwadge chain were selected for this study on the basis of accessibility, preliminary sample collections, and historic water and sediment quality. All of the lakes were similar in terms of mean depth and surface area. Wowun (WOW) and Loken (LOK) lakes were chosen as the reference sites (Table 1). Manitouwadge (MAN), Mose (MOS), Little Manitouwadge (LMN), and Little Mose (LMO) lakes were selected as the most contaminated water bodies, while Lakes Kaginu (KAG) and Agonzon (AGO), located further downstream from the mines, provided sites of intermediate contamination.

Fish were collected from each of the eight lakes in August 1988, using both nylon-monofilament and cotton-twine gill nets. Stretched mesh sizes ranged from 7.5 to 10.0 cm. Nets were set in the evening between 19:00 and 21:00 and emptied

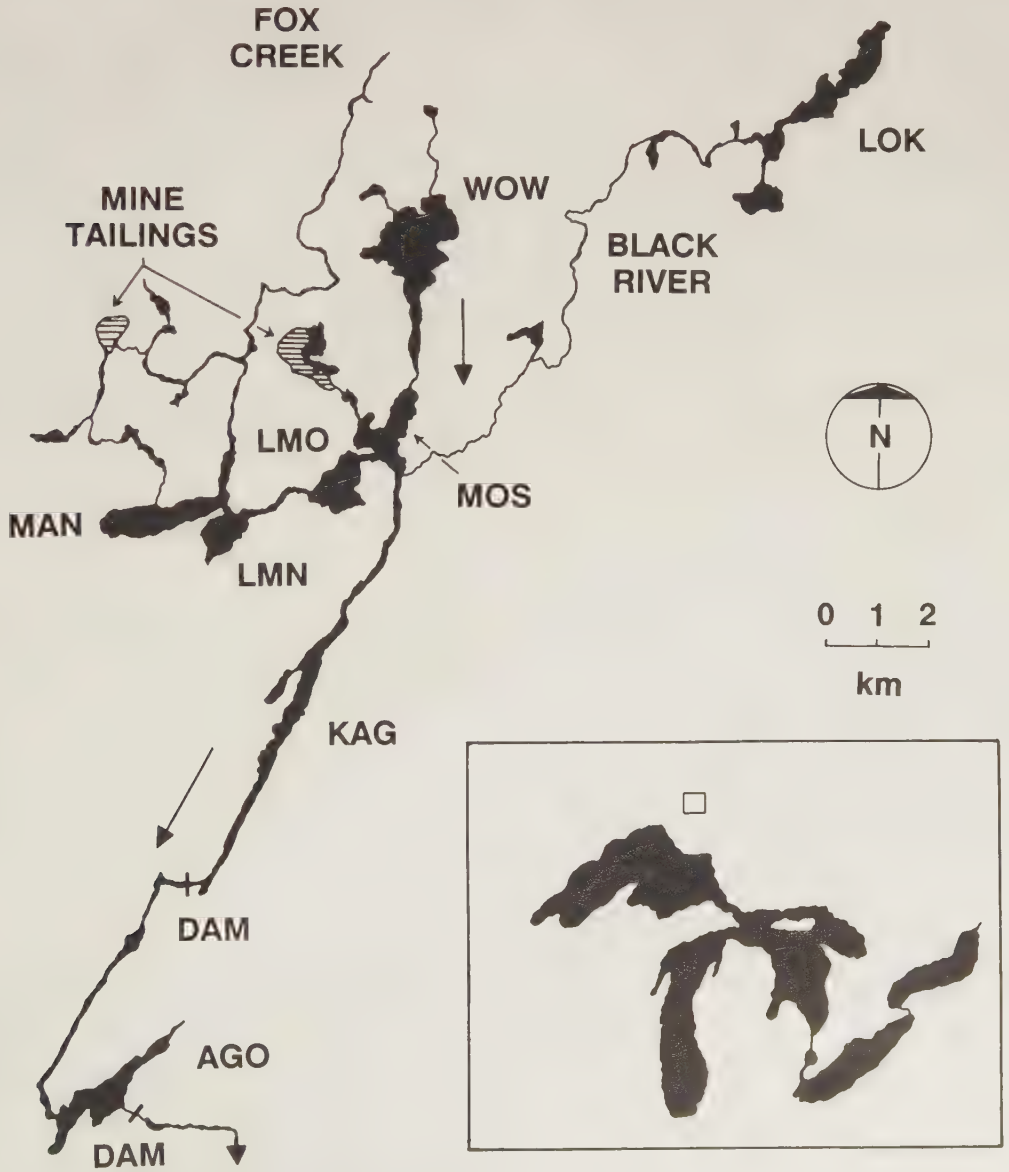

FIG. 1. Map of the study site showing the location of Wowun (WOW), Loken (LOK), Manitouwadge (MAN), Little Manitouwadge (LMN), Mose (MOS), Little Mose (LMO), Kaginu (KAG), and Agonzon (AGO) lakes. Insert shows location relative to the Great Lakes.

TABLE 1. Mean (SE, n) concentrations of Cu and Zn in water and geometric mean concentrations in sediment and invertebrate samples collected in the area of the fish nets. Within columns, means with a common superscript are not significantly different ($p > 0.01$).

	Water ($\mu g \cdot L^{-1}$)		Sediment ($mg \cdot kg^{-1}$)		Invertebrates ($mg \cdot kg^{-1}$)	
Lake	Zn	Cu	Zn	Cu	Zn	Cu
MAN	156 (3, 8)[a]	15 (0.4, 8)[a]	6397 (539, 8)[a]	93 (14, 8)[a]	527 (134, 4)[a]	89 (19, 4)[a,b]
LMN	50 (3, 8)[b]	11 (0.7, 8)[a,b]	1107 (320, 8)[a,b]	28 (5, 8)[b,c]	305 (53, 4)[a,b]	189 (34, 4)[a]
MOS	42 (3, 8)[b,c]	10 (0.4, 8)[a,b]	2455 (499, 8)[a,b]	43 (13, 8)[a,b,c]	377 (247, 4)[a,b]	125 (32, 4)[a,b]
LMO	39 (4, 8)[b,c]	6 (0.4, 8)[b,c,d]	1435 (427, 8)[a,b]	58 (14, 8)[a,b]	132 (76, 4)[a,b]	57 (31, 4)[a,b]
KAG	35 (2, 8)[c]	9 (0.7, 8)[b,c]	1897 (152, 8)[a,b]	71 (10, 8)[a,b]	502 (195, 4)[a,b]	103 (30, 4)[a,b]
AGO	12 (0.4, 8)[d]	4 (2, 8)[c,d]	286 (102, 8)[b]	21 (4, 8)[c]	460 (84, 4)[a,b]	61 (17, 4)[a,b]
LOK	4 (0.7, 8)[d]	4 (0.4, 8)[c,d]	11 (4, 8)[d]	6 (2, 8)[d]	35 (12, 4)[a,b]	99 (9, 4)[a,b]
WOW	5 (0.7, 8)[d]	2 (1, 8)[d]	27 (5, 8)[c]	3 (0.4, 8)[d]	29 (7, 4)[b]	57 (6, 4)[b]

in the morning from 06:00 to 09:00. Fish were transported to the shore on ice, killed by a blow to the head, and sampled as quickly as possible. Weight and standard length were recorded for each fish. Condition factor (k) was calculated as 100 (weight/standard length^3). The left operculum and a pectoral fin ray were removed from each fish for age determination.

Small portions of liver, kidney, gill, bone, axial muscle, and reproductive tissue were removed from each fish. The muscle sample was removed from the left side of the fish anterior to the dorsal fin and posterior to the operculum. Digesta were sampled for six suckers of each sex, from the stomach, the anterior 5 cm of the intestine, the 5-cm portion of the intestine posterior to the loop, and the remaining posterior portion of the intestine. All samples were immediately frozen in liquid nitrogen (−196°C), transported to the laboratory, and stored at −20°C pending metal analysis.

Four filtered (0.45 μm) water samples were obtained at each of two sites for each lake, based on gillnet location. The samples were taken 25–30 cm below the surface and preserved with 1 mL of reagent-grade 15.9 M nitric acid per 100 mL of water.

Sediment samples were taken in 0.5–1.0 m of water at the same sites as the water samples. Samples were taken from the top 5 cm of the sediment using a four-sample sediment corer (Freshwater Biological Association design; area, 19.6 cm^2), with each core having a diameter of 5 cm. Four samples from each site were held on ice for transport and then immediately frozen in liquid nitrogen. Invertebrate samples were taken at each site (in 0.5–1.0 m of water) using the sediment corer. Each sample was emptied into a net, agitated in water to release excess sediment, and stored in 10% formalin pending metal analysis.

Metal levels in water, fish tissue, invertebrate, and sediment samples were determined by flame-volatilization atomic absorption spectrophotometry after appropriate preparation. Water samples were analyzed by direct aspiration. Fish tissue samples were oven-dried for 60 h at 60°C, weighed, and transferred to 125-mL erlenmeyer flasks. Mixed acid (1:8, v/v, 18 M H_2SO_4:15.9 M HNO_3) was added to the flasks at a rate of 5 mL per 0.3–0.4 g of sample. The samples were refluxed using small glass funnels at 200–220°C for approximately 1 h with the addition of 5 mL of hydrogen peroxide (30%) and then, prior to analysis, diluted to either 25 or 50 mL with distilled water. Invertebrates were analyzed using the protocol applied to fish tissue, with the exception that replicate samples were pooled (two per pool) to increase sensitivity. Sediment samples were freeze-dried to constant weight but not sieved (Poulton et al. 1988). Two- to 4-g aliquots were shaken overnight in 50 mL of 0.5 N HCl (R. Agemian, Environment Canada, National Water Research Institute, Canada Centre for Inland Waters, Burlington, Ont. L7R 4A6, pers. comm.), centrifuged, and the supernatant diluted prior to analysis.

All analyses were completed with a Perkin-Elmer 4000 atomic absorption spectrophotometer equipped with an impact bead to increase the sensitivity (detection limits: Cu, 1 $\mu g \cdot L^{-1}$; Zn, 1 $\mu g \cdot L^{-1}$). Standard curves of Cu and Zn concentration were constructed using commercially available certified stock solutions. Analytical blanks appropriate to the design were completed in conjunction with the samples. The method for determining the levels of Cu and Zn in tissues was verified with National Bureau of Standards bovine liver (lot 1577a). Our isolation method yielded mean values of 156.1 ± 3.7 $\mu g\ Cu \cdot g^{-1}$ and 117.8 ± 6.7 $\mu g\ Zn \cdot g^{-1}$, representing, respectively, 99 and 96% recovery.

Data were plotted to confirm homogeneity of variance prior to analysis. Sediment, invertebrate, and all fish tissue data were log-transformed for further analysis and are reported as geometric means. All tissue data were analyzed using SYSTAT to perform a two-way factorial analysis of variance with lake and sex as main-effects factors. Invertebrate data were analyzed by lake using a one-way analysis of variance. For the analyses of variance, differences were deemed significant at $p \leq 0.01$, since variability in the field required a more conservative significance level to distinguish between natural fluctuation and actual site differences. Significant differences were analyzed further using Tukey's multiple comparison test. After completion of the above, the means were ranked for the development of a Spearman's correlation matrix. Only those means which showed a statistically significant lake effect during analysis of variance were ranked. The statistical significance of the correlations is reported at both the $p \leq 0.01$ and $p \leq 0.05$ levels.

Results

Analysis of variance revealed a significant difference among lakes in the concentrations of Cu and Zn in water ($p < 0.001$ for both), sediments ($p < 0.001$ for both), and invertebrates (Cu, $p = 0.005$; Zn, $p = 0.001$) (Table 1). In most cases the concentrations relating to MAN were a great deal higher than those in the other lakes. LMN was usually the next highest, while all other lakes had decreasing concentrations down to the background levels of the two reference lakes, WOW and LOK. Aside from the two reference sites, waterborne Zn levels were lowest in AGO and increased with proximity to the mine site:

TABLE 2. Mean standard length (SE, $n = 12$), weight (SE, $n = 12$), condition factor (k) (SE, $n = 12$), and age (SE, n) of white sucker from the eight study lakes. Within columns, means with a common superscript are not significantly different ($p > 0.01$).

Lake	Standard length (cm)	Weight (g)	Age (yr)	k
MAN	32 (0.7)^{c,d}	636 (44)^{c,d}	9 (0.9, 9)^{b,c}	1.84 (0.03)^{a,b,c,d}
LMN	36 (0.7)^{a,b,c,d}	781 (40)^{b,c,d}	10 (1, 10)^{b,c}	1.66 (0.03)^{c,d}
MOS	41 (0.7)^{a}	1138 (38)^{a}	15 (0.5, 13)^{a}	1.65 (0.06)^{d}
LMO	38 (1.0)^{a,b}	965 (70)^{a,b}	12 (0.9, 12)^{a,b}	1.76 (0.03)^{a,b,c,d}
KAG	37 (1.2)^{a,b,c}	910 (77)^{a,b,c}	12 (0.8, 9)^{a,b}	1.72 (0.03)^{b,c,d}
AGO	35 (1.4)^{b,c,d}	848 (114)^{a,b,c,d}	8 (2.4, 4)^{b,c}	1.88 (0.06)^{a,b,c}
LOK	33 (0.6)^{b,c,d}	700 (34)^{b,c,d}	6 (0.3, 9)^{c}	1.93 (0.03)^{a,b}
WOW	31 (0.8)^{d}	597 (52)^{d}	8 (0.5, 11)^{b,c}	1.96 (0.03)^{a}

TABLE 3. Mean (SE, n) Cu concentrations (mg·kg $^{-1}$) in tissues of white sucker from the study lakes. Within columns, means with a common superscript are not significantly different ($p > 0.01$). Digesta = stomach contents.

Lake	Liver	Kidney	Gill	Ovary	Testis	Muscle	Bone	Digesta
MAN	98 (8, 11)^{a}	31 (4, 6)^{a}	9 (2, 8)^{a}	13 (1, 5)^{a}	10 (3, 3)^{a}	5 (1, 6)^{a}	4 (0.7, 8)^{a}	107 (60, 6)^{a}
LMN	94 (12, 7)^{a,b}	29 (4, 8)^{a}	8 (1, 8)^{a}	6 (0, 1)^{a}	3 (1, 4)^{b}	3 (1, 8)^{b}	4 (1.1, 8)^{a}	51 (18, 7)^{a,b}
MOS	79 (10, 7)^{a,b}	13 (1, 8)^{b}	6 (1, 8)^{a,b}	10 (2, 4)^{a}	4 (1, 4)^{a,b}	3 (0.4, 8)^{a,b}	3 (0.5, 6)^{a}	19 (2, 6)^{b,c}
LMO	83 (19, 7)^{a,b}	12 (1, 8)^{b}	5 (1, 8)^{a,b}	10 (2, 3)^{a}	4 (1, 3)^{a,b}	5 (1, 8)^{a,b}	4 (0.5, 6)^{a}	39 (11, 7)^{a,b}
AGO	84 (9, 7)^{a,b}	11 (0.4, 8)^{b}	3 (1, 8)^{b}	13 (2, 6)^{a}	3 (1, 2)^{a,b}	3 (0.4, 8)^{b}	5 (0.2, 4)^{a}	23 (3, 6)^{a,b,c}
KAG	67 (10, 7)^{a,b}	9 (1, 8)^{b}	5 (0, 8)^{a,b}	13 (1, 2)^{a}	2 (1, 4)^{b}	3 (0.4, 7)^{a,b}	4 (0.5, 6)^{a}	19 (7, 7)^{b,c}
LOK	51 (6, 12)^{b}	8 (0.7, 8)^{b}	6 (1, 8)^{a,b}	13 (3, 3)^{a}	3 (1, 5)^{a,b}	4 (0.4, 7)^{a,b}	4 (0.8, 6)^{a}	5 (3, 6)^{c}
WOW	46 (9, 7)^{b}	10 (1, 10)^{b}	3 (2, 5)^{b}	8 (1, 4)^{a}	2 (0, 5)^{b}	3 (0.4, 8)^{a,b}	3 (0.1, 7)^{a}	16 (1, 6)^{b,c}

AGO < KAG < LMO < MOS < LMN < MAN. Zinc levels were consistently higher than Cu levels, and MAN showed almost a threefold increase in Zn levels in water and sediment over all other sites (Table 1). Cu levels were also highest at MAN.

There were no effects of gender on mean weight ($p = 0.056$), standard length ($p = 0.069$), age ($p = 0.442$), or k ($p = 0.999$) of white sucker from the eight sites (Table 2). The samples were pooled for further analysis of the parameters. There were large differences among lakes in the age, length, and weight of white sucker ($p < 0.001$). This effect of lake was further analyzed using Tukey's multiple comparison test. The differences in age, length, and weight between lakes showed no apparent correlations with the gradient of metal contamination. There was also a large difference in k among lakes ($p < 0.001$). In this case, however, Tukey's test showed a gradual shift in the condition of the fish, with the white sucker from the least contaminated and reference lakes having the highest k (Table 2).

With respect to Cu concentrations, only those in liver ($p = 0.001$) and gonad ($p < 0.001$) were affected by gender. For both tissues, the concentrations of Cu in the female fish were higher than those in the males. The concentration of Cu in the testes was significantly different among fish from different lakes (Table 3), although the levels were not related to the degree of water or sediment contamination. There was no lake effect on ovary Cu level. Relative to the reference lakes, white sucker from lakes with elevated levels of Cu in water and sediments also had higher Cu levels in their other tissues. There was a significant difference among lakes in the concentration of Cu in liver ($p = 0.001$), kidney ($p < 0.001$), and gill ($p = 0.001$) tissue. There was also a significant difference in muscle Cu among lakes ($p = 0.002$), although the differences were not correlated with water and sediment metal concentrations (Table 3). There was no difference in Cu concentration in bone ($p = 0.988$) among lakes. There was a significant lake effect on the Cu levels in stomach contents ($p < 0.001$; Table 3); the same relationship was apparent for the digesta from the other portions of the gastrointestinal tract (Miller 1990).

Trends similar to Cu were noted for Zn concentrations in the various tissues. There was a large difference between sexes in the concentration of Zn in gonad tissue ($p < 0.001$). Again, the concentrations in ovaries were higher than those in testes, although when they were analyzed separately, there were no significant differences among lakes (Table 4). There were sig-

TABLE 4. Mean (SE, n) Zn concentrations (mg·kg $^{-1}$) in tissues of white sucker from the study lakes. Within columns, means with a common superscript are not significantly different ($p > 0.01$). Digesta = stomach contents.

Lake	Liver	Kidney	Gill	Ovary	Testis	Muscle	Bone	Digesta
MAN	275 (40, 11)^{a}	269 (13, 6)^{a}	117(13, 8)^{a}	210 (26, 5)^{a}	138 (23, 4)^{a}	29 (5, 6)^{a}	288 (42, 8)^{a}	1230 (372, 6)^{a}
LMN	240 (61, 7)^{a,b}	200 (16, 8)^{a,b}	93 (8, 8)^{a,b}	165 (0, 1)^{a}	74 (8, 5)^{a}	19 (4, 8)^{a}	174 (22, 8)^{a,b}	661 (183, 7)^{a,b}
MOS	158 (9, 7)^{a,b,c}	148 (9, 8)^{b,c}	87 (6, 8)^{a,b}	117 (10, 4)^{a}	48 (15, 4)^{a}	21 (4, 8)^{a}	132 (1, 8)^{a,b,c}	257 (29, 6)^{a,b,c}
LMO	145 (11, 7)^{b,c}	135 (7, 8)^{c}	81 (4, 8)^{b}	112 (11, 3)^{a}	78 (11, 3)^{a}	26 (5, 7)^{a}	129 (8, 6)^{a,b,c}	380 (103, 7)^{a,b}
AGO	151 (12, 7)^{b,c}	138 (5, 8)^{b,c}	78 (4, 8)^{b}	101 (4, 2)^{a}	63 (2, 3)^{a}	20 (5, 8)^{a}	91 (14, 4)^{b,c}	229 (38, 6)^{a,b,c}
KAG	117 (12, 7)^{c}	120 (9, 8)^{c}	68 (2, 8)^{b}	109 (4, 2)^{a}	78 (4, 4)^{a}	18 (5, 7)^{a}	83 (7, 7)^{b,c}	123 (51, 7)^{a,b,c}
WOW	123 (8, 7)^{c}	117 (5, 10)^{c}	74 (3, 5)^{b}	159 (46, 4)^{a}	60 (4, 5)^{a}	22 (5, 7)^{a}	58 (4, 7)^{c}	102 (7, 6)^{b,c}
LOK	120 (9, 12)^{c}	112 (3, 8)^{c}	72 (2, 8)^{b}	131 (18, 3)^{a}	59 (11, 5)^{a}	21 (4, 7)^{a}	59 (5, 6)^{c}	24 (17, 6)^{c}

TABLE 5. Spearman's correlation coefficient matrix for Cu concentrations in several environmental and fish tissue compartments in each lake. Condition factor (k) is also included. The means from each lake were ranked prior to analysis. The critical values for statistical significance are 0.833 at $p \leq 0.01$ (superscript a) and 0.643 at $p \leq 0.05$ (superscript b).

	Environmental metal levels				Tissue metal levels				
	Invertebrates	Water	Sediment	k	Liver	Kidney	Gill	Muscle	Stomach
Invertebrates	1.000								
Water	0.857[a]	1.000							
Sediment	0.595	0.786[b]	1.000						
k	−0.952[a]	−0.738[b]	−0.571	1.000					
Liver	0.548	0.786[b]	0.619	−0.429	1.000				
Kidney	0.575	0.790[b]	0.479	−0.479	0.874[a]	1.000			
Gill	0.539	0.743[b]	0.443	−0.407	0.491	0.627	1.000		
Muscle	−0.191	0.218	0.436	0.218	0.355	0.247	0.508	1.000	
Stomach	0.563	0.790[b]	0.719[b]	−0.419	0.958[a]	0.765[b]	0.458	0.358	1.000

TABLE 6. Spearman's correlation coefficient matrix for Zn concentrations in several environmental and fish tissue compartments in each lake. Condition factor (k) is also included. The means from each lake were ranked prior to analysis. The critical values for statistical significance are 0.833 at $p \leq 0.01$ (superscript a) and 0.643 at $p \leq 0.05$ (superscript b).

	Environmental metal levels				Tissue metal levels				
	Invertebrates	Water	Sediment	k	Liver	Kidney	Gill	Bone	Stomach
Invertebrates	1.000								
Water	0.548	1.000							
Sediment	0.786[b]	0.810[b]	1.000						
k	−0.690[b]	−0.714[b]	−0.667[b]	1.000					
Liver	0.190	0.786[b]	0.500	−0.357	1.000				
Kidney	0.500	0.905[a]	0.714[b]	−0.571	0.929[a]	1.000			
Gill	0.143	0.833[a]	0.548	−0.405	0.976[a]	0.905[a]	1.000		
Bone	0.476	0.976[a]	0.738[b]	−0.643	0.881[a]	0.952[a]	0.905[a]	1.000	
Stomach	0.357	0.929[a]	0.714[b]	−0.548	0.857[a]	0.929[a]	0.905[a]	0.952[a]	1.000

nificant differences among lakes in the concentrations of Zn in the liver ($p < 0.001$), kidney ($p < 0.001$), gill ($p < 0.001$), bone ($p < 0.001$), and stomach contents ($p < 0.001$) (Table 4). The differences among lakes in muscle Zn levels were marginal ($p = 0.018$).

After completion of the analysis outlined above, the sediment, water, invertebrate, condition factor, and tissue data were subjected to a Spearman's correlation test to determine the relative importance of diet (through invertebrates), sediments, and water to the concentrations of Cu and Zn in the various tissues of white sucker (Tables 5 and 6). For both metals the majority of significant correlations concerned water, rather than sediment or invertebrates.

The concentrations of Cu in invertebrates were significantly correlated with waterborne but not with sediment Cu concentration (Table 5). For Zn the trend was reversed; Zn concentrations in the invertebrates were correlated with sediment but not with waterborne Zn concentrations (Table 6). The concentrations of Cu and Zn in fish tissues were consistently more strongly correlated with waterborne metal levels than with those in sediments. There were no significant correlations between tissue metal and invertebrate metal concentrations (Tables 5 and 6). The concentrations of Zn in the liver, kidney, gill, bone, and stomach were all significantly correlated with waterborne Zn concentrations. In addition, the concentrations in kidney, bone, and stomach were, to a lesser extent, correlated with sediment Zn levels (Table 6). The Zn concentrations in these tissues were also correlated with each other. In simple terms, when Zn was elevated in the liver, it was also elevated in the kidney, gill, bone, and stomach. Cu concentrations in liver, kidney, gill, and stomach were correlated with Cu concentration in water. Cu concentration in the stomach was marginally correlated with sediment Cu concentration (Table 5). The concentration of Cu in the liver was significantly correlated with the Cu concentration in both the kidney and the stomach contents.

The k was significantly correlated with Cu levels in invertebrates and water, as well as with Zn levels in invertebrates, sediments, and water. The correlation between k and invertebrate Cu level was the strongest obtained.

Discussion

The Manitouwadge lakes provided an excellent gradient of metal contamination for our study on the bioavailability of Cu and Zn to white sucker. Levels of Cu and Zn were elevated in both the water and sediment at the contaminated sites, a situation reflected in the metal levels in invertebrates and fish tissues. The concentrations of Cu in the invertebrates were more strongly correlated with water ($r = 0.857$) than with sediment ($r = 0.595$), suggesting that, relative to sediment, water plays a dominant role for Cu accumulation in invertebrates. The trend was reversed for Zn, with the concentration in invertebrates more strongly correlated with sediment ($r = 0.786$) than with water ($r = 0.548$), suggesting that sediments are the predominant contributor to invertebrate Zn levels.

The Cu concentrations in white sucker tissues were significantly correlated with Cu concentrations in water but not with those in sediments or invertebrates. A similar trend was noted for Zn. The relationship between water and tissue concentrations was stronger than for Cu, although kidney and bone Zn concentrations were also significant. These data indicate that

aqueous Cu and Zn concentrations are reasonable predictors of metal levels in white sucker tissues, while the role of invertebrates would seem to be negligible.

Despite the poor correlations between both sediment and invertebrate metal levels and tissue metal levels in fish, the levels of metals in the stomach contents of the white sucker suggest that this may represent an entry point for these metals. Metal levels in plankton and invertebrates are commonly higher than in the fish which feed upon them (German 1971; Jackson et al. 1980; Heit and Klusek 1984). Dietary input can represent a major source of metal uptake (Patrick and Loutit 1976; Dallinger and Kautzky 1985), and in young plaice the diet can be the major source of essential Zn up to water concentrations of 600 $\mu g \cdot L^{-1}$ (Milner 1982). When interpreting the invertebrate metal data reported here, it should be remembered that we were solely interested in the contribution of the invertebrate food resource to the dietary metal levels in white sucker. Since some invertebrates ingest and pass sediment through their gastrointestinal tract (Cairns et al. 1984), analyzing metal concentrations of invertebrates without allowing time for gut clearance can lead to erroneously high estimates of body concentration, from the invertebrate point of view (Hare et al. 1989). This was of no consequence in our study because white sucker consume the organisms whole. In addition, we did not separate and analyze invertebrate species individually, but pooled them by site, and the species of invertebrates present differed from site to site. Invertebrate species vary widely in their tolerance of metals (Chapman et al. 1980), as well as in the metabolic strategy associated with that tolerance. Some accumulate high metal levels, while others are extremely efficient at metal excretion (Patrick and Loutit 1976; Niederlehner et al. 1984; O'Grady and Abdullah 1985). Separate analysis of invertebrate species, although not within the scope of this study, would have given a more accurate picture of the relative uptake of metals by benthic organisms (Krantzberg and Stokes 1988).

Cu and Zn levels in liver and kidney were, relative to most other tissues, reliable indicators of chronic Cu and/or Zn exposure. Both liver and kidney represent sites of metal metabolism and excretion. Increases in Cu and Zn in metabolic tissues have been previously reported (German 1971; Roch and McCarter 1984). Abnormally high levels of these two metals in the liver have been shown to induce metallothionein production (Cousins 1985). The concentrations of Zn in bone tissue were significantly elevated in fish collected from MAN and LMN. Bone Zn was correlated with both water and sediment Zn concentrations, although the correlation was stronger (1%) for water than it was for sediment (5%). Mount (1964) exposed bluegill sunfish (*Lepomis macrochirus*) to sublethal concentrations of Zn for 90 d and found elevated concentrations of Zn in opercular bone. Zn is involved in normal bone formation (Cousins 1985), and O'Grady and Abdullah (1985) found Zn to be concentrated in skeletal and liver tissue. In skeletal tissue, the deposition of Zn was incorporated in the highly insoluble calcium phosphate matrix and was therefore not easily mobilized. This lack of mobility contributes to the strength of the correlations between environmental and bone Zn level and hence to the utility of bone Zn as an indicator of long-term contamination (Mount 1964).

The concentrations of Cu and Zn in white sucker muscle were not related to environmental exposure. Although the concentrations of Cu in the muscle differed among lakes, there was no consistent trend among muscle Cu and the concentration of Cu in water and sediment. Although metals such as mercury (Hg) and cadmium (Cd) are found at elevated levels in muscle, it appears that normal homeostatic mechanisms for Cu and Zn allow partial isolation of the muscular compartment from environmental elevations (Cross et al. 1973; Wiener and Giesy 1979; Wilson 1980). Of the tissues sampled in this study, muscle was the poorest indicator of environmental exposure to Cu and Zn.

In a previous study, Munkittrick and Dixon (1988) evaluated growth, fecundity, and energy stores in white sucker from MAN relative to LOK lakes. The MAN population showed reduced growth in females after sexual maturation, decreased egg size and fecundity, no significant increase in fecundity with age, and an increased incidence of spawning failure. They suggested that the failure of female fish to grow significantly after maturity and the decreased energetic commitment to reproduction were probably the result of a decrease in available food rather than a direct effect of metal accumulation. The significant correlations between the k of white sucker and metal concentrations in invertebrates (for both Cu and Zn) reported here may support this conclusion. Given that elevated levels of environmental metals will reduce both the diversity and abundance of benthic invertebrates (Chapman et al. 1980), the correlations between k and benthos metal levels may reflect (indirectly) the decreased quality of the white sucker's food resource. This is strengthened by the lack of significant correlations between Cu and Zn levels in benthos and white sucker tissue.

In conclusion, Cu concentrations in benthic invertebrates were correlated with water concentrations and not with sediment concentrations. This trend was reversed for Zn, where the sediment concentration was a better predictor of invertebrate contamination than the concentration of Zn in water. The negative correlation between k and benthos metal levels and the lack of a significant correlation between benthos and tissue metal levels suggest an indirect effect of metal contamination of invertebrates on white sucker. Most fish tissues from the contaminated sites showed elevated levels of both Cu and Zn relative to the reference sites. Liver and kidney were reliable indicators of chronic Cu and Zn contamination, and Zn exposure was also reflected in bone tissue. Muscle was a poor indicator of low-level Cu and Zn contamination. For both metals, the water concentration was a better predictor of fish tissue metal level than were the concentrations in either the sediment or the invertebrates used as a food source.

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Spatial Aggregation, Precision, and Power in Surveys of Freshwater Mussel Populations[1]

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We studied aggregation in 76 populations of freshwater mussels from relatively homogeneous surroundings in a wide range of habitats. Chi-square tests for spatial aggregation found only 53% of mussel populations significantly ($p < 0.05$) aggregated. The variance of replicate mussel samples (s^2) varied with the mean number collected (m) as $1.49m^{1.17}$, but conformed to the general variance relation found for other aquatic taxa ($m^{1.5}$) at $m > 1$. The number of replicate samples ($\hat{n}$) required to estimate mussel abundance with a given level of precision ($D = \text{SE}/m$) is approximately $m^{-0.5}D^{-2}$. Sampling mussels with large quadrats requires between 5 and 25 samples for 20% precision. Sampling designs to determine significant impacts ($\alpha = \beta = 0.05$) require 7–50 samples of each population to detect doubling or halving of the population density, or three to nine to detect order-of-magnitude changes. Large sampling units are recommended to ensure acceptable sampling precision and accurate chi-square analyses of spatial aggregation and to permit ecologists to detect significant impacts on freshwater mussel populations.

Nous avons étudié l'agrégation chez 76 populations de moules d'eau douce vivant dans des milieux relativement homogènes et provenant d'une vaste gamme d'habitats. Des tests de khi carré de l'agrégation spatiale nous ont permis de trouver que seulement 53 % des populations de moules étaient agrégées de façon significative ($p < 0,05$). La variance des réplicats d'échantillons de moules (s^2) variait avec le nombre moyen de moules recoltées (m) soit : $1,49m^{1,17}$, mais suivait la relation générale de variance trouvée pour d'autres taxa aquatiques ($m^{1,5}$) lorsque $m > 1$. Le nombre d'échantillons nécessaires ($\hat{n}$) pour estimer l'abondance des moules avec un niveau de précision donné ($D = \text{SE}/m$) est d'environ $m^{-0,5}D^{-2}$. L'échantillonnage des moules à l'aide de grands quadrats nécessite la récolte de 5 à 25 échantillons afin d'obtenir une précision de 20 %. Il faut une plus grande réplication lorsqu'il est nécessaire de déterminer des impacts significatifs ($\alpha = \beta = 0,05$) : de 7 à 50 échantillons de chaque population pour détecter une densité de population qui double ou diminue de moitié, ou de 3 à 9 échantillons pour déterminer des changements d'ordre de grandeur. L'utilisation de grandes unités d'échantillonnage est recommandée afin d'assurer une précision d'échantillonnage acceptable, l'exactitude des analyses d'agrégation spatiale avec le khi carré et afin de permettre aux écologistes de détecter des impacts significatifs sur les populations de moules d'eau douce.

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The study of populations of freshwater mussels is of growing importance. Unionid mussels are gaining prominence in monitoring and detecting pollution (Day et al. 1990; Metcalfe and Charlton 1990) and acidification (Pynnönen 1990). They form the basis of the world's freshwater pearl industry (Coker et al. 1922; Kat 1982). Unionid glochidia can be fatal parasites of sport fishes (Lefevre and Curtis 1910; Matteson 1948), and juveniles are important food for fish (Negus 1966) and small mammals (Coker et al. 1922; Cvancara 1970). Freshwater mussels may have a major impact on phytoplankton dynamics and lake management because their feeding is unselective (Winter 1978; Tessier et al. 1984) and filtering rates are high (Price and Schiebe 1978). Mussels can make up much of the benthos biomass in lakes (Magnin and Stanczykowska 1971; Golightly and Kosinsky 1981), affect alkalinity budgets (Green 1980), and stimulate microinvertebrate production (Sephton et al. 1980). They are of paleontological (Green 1972) and archaeological (Kunz 1893; Hill 1983) interest. Many species are now endangered (Strayer 1980; DiStephano 1984; Miller et al. 1986) by habitat modification and the introduction of exotics like the Asian clam (*Corbicula fluminea*) (Lauritsen and Mozley 1989; Leff et al. 1990) and the zebra mussel (*Dreissena polymorpha*) (Hebert et al. 1989).

In spite of the broad importance of unionid population studies, sampling difficulties engendered by their spatial heterogeneity have made it difficult to make precise estimates of their abundance and study their population dynamics (Kat 1982). Although sampling programs for other benthic faunae can be planned and refined through the use of empirical sampling algorithms predicting natural spatial heterogeneity, no such treatment exists for freshwater mussels, and existing analyses of sampling design (e.g. Downing 1979) may not be applicable to them (see Riddle 1989). Ecologists thus have no systematic means of planning programs to sample freshwater mussels, and quantitative data on unionid population density remain scarce.

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Smaller benthic invertebrates are known to be aggregated in space (Elliott 1977; Downing 1979). Usually, the variance (s^2) of replicate samples of benthic invertebrates is found to be significantly greater than the average of replicate counts (m). Downing (1979) found for lake benthos that the "variance was less than the mean in only 2.5% of 1500 sets of data examined." The biology of unionid molluscs might lead them to be more aggregated than other invertebrate groups. Among the most frequently and earliest cited causes of spatial aggregation is its possible value for reproduction (e.g. Anscombe 1950). The frequency of hermaphroditism in unionid populations (e.g. van der Schalie 1970) indicates that fertilization might be a difficult stage in their life cycle (Ghiselin 1974). Animals such as freshwater mussels, living at low densities and employing direct fertilization, might improve reproduction by clumping. On the other hand, low rates of predation in freshwater mussel populations might lead them to be randomly distributed (Rasmussen and Downing 1988). Observations made over the last 80 yr on the spatial heterogeneity of unionid molluscs (Lefevre and Curtis 1910; Little and Gentner 1969; Kessler and Miller 1978; Sephton et al. 1980; Mitchell and Collins 1984) have suggested that populations are aggregated, but the frequency of aggregation in this group is unknown.

Research on other groups of benthic invertebrates has not only shown that spatial distributions are aggregated, but that empirical observations of spatial s^2 of replicate samples rises with m in a predictable fashion (Downing 1979; Morin 1985; Vézina 1988). In general, s^2 of benthos samples varies as

$$(1) \quad s^2 = am^b$$

where a and b are fitted coefficients and b is usually between 1 and 2 (Downing 1979; Morin 1985; Vézina 1988). Further, a recent review of the literature on spatial variation in aquatic organisms (Downing 1991) suggests, on the basis of over 18 000 sets of replicate samples, that variance algorithms for aquatic taxa tend to

$$(2) \quad s^2 = m^{1.5}$$

on average. If eq. 1 or 2 holds for unionid mussels, then the number of requisite samples ($\hat{n}$) needed to obtain a given level of precision ($D = \text{SE}/m$) could be estimated a priori (Downing 1979; Cyr et al. 1992):

$$(3) \quad \hat{n} = a \cdot m^{b\ -2} D^{-2} .$$

The effectiveness of such a strategy for the optimization of sampling programs is now well established (McIntyre et al. 1984; Morin 1985; Vézina 1988; Downing 1989), saving researchers wasted sampling effort and failed surveys, but is unavailable for unionid mussels.

If freshwater mussels are highly aggregated in space, the high variance of population samples may lead sampling surveys to be inconclusive (i.e. low power) (Forbes 1990; Peterman 1990). This will become increasingly important as sampling surveys are designed to monitor the effects of pollution or competition between exotic and indigenous species. If spatial heterogeneity of unionid populations is not accounted for in designing sampling surveys, we may be unable to conclude that there have been significant influences of exotic species like *Corbicula* or *Dreissena* until indigenous unionid populations have been completely replaced by exotics.

This article has two objectives. First, we test the hypothesis that unionid mussel populations are aggregated in space and that they are aggregated as frequently as other aquatic taxa. Second, we examine the predictability of spatial variance and show how this information can be used in planning precise and powerful surveys of freshwater mussel abundance.

Methods

The hypothesis that unionid mollusc populations are aggregated in space was tested in 76 populations (Table 1). Only populations that were found in relatively homogeneous surroundings were considered in order to avoid the biasing effects of sampling across gradients or among sampling strata (Downing 1991). Data on the mean number of mussels (m) collected per randomly or regularly placed sampling unit and the $n - 1$ weighted variance of these means (s^2) were derived from original collections or from the published literature. Because of the paucity of published quantitative data on unionid population density (cf. Vézina 1988), we made specific collections of mussel populations in northern Minnesota and southern Québec, covering ultraoligotrophic to eutrophic lakes across a representative range of habitat types. For each population, a chi-square test was applied to s^2/m to test for agreement with a Poisson distribution (Elliott 1977). A s^2:m ratio significantly greater than 1 indicates that the population was significantly aggregated.

The hypothesis that spatial s^2 of unionid populations follows eq. 1 was tested by least squares regression analysis (Draper and Smith 1981) of the data in Table 1. Data were $\log_{10}$ transformed to linearize the response. The quantitative equivalence of the spatial heterogeneity of unionid molluscs and other aquatic taxa was tested by examining the fit of the spatial variances for unionids (Table 1) to the average variance function for all aquatic taxa (eq. 2) calculated from the data reviewed by Downing (1991). Variance functions were used to calculate the number of samples needed for a given level of precision ($\hat{n}$) following the protocol outlined by Downing and Anderson (1985) as modified by Downing (1989).

Past studies of sampling design have been oriented toward improving the precision of population estimates, but environmental impact studies must not only be precise but powerful enough to detect specified levels of change in population density. In studying the potential impact of exotic species on indigenous ones, the ability to determine significant differences among average densities of unionid mussel populations does not depend only on the precision of each mean. A given level of precision may or may not be adequate, depending on the size of difference that one needs to be able to detect. In such cases, power analysis can be used to optimize the sampling design (Cohen 1988; Peterman 1990).

Measures of spatial aggregation can be used to calculate the number of samples required to detect a given difference between two mean population densities using a t-test (Cyr et al. 1992). The analysis sought the number of samples needed to provide t-tests for differences between hypothetical means (m_1, m_2),

$$(4) \quad t = \frac{m_1 - m_2}{s'_{m_1 - m_2}} ,$$

that would be statistically significant at a given level of α and β. The number of samples ($n = n_1 = n_2$) necessary to reach a given level of α and β (where β is $1 -$ power) was determined iteratively for pairs of m_1 and m_2 where m_1 and m_2 varied between 1 and ≈300 mussels per sample. The sample numbers n_1 and n_2 were set equal to each other in this analysis for com-

TABLE 1. Data used for the analysis of spatial aggregation in unionid mussels. Minnesota lake data were collected in Clearwater Creek (47°22′N, 93°25′W), Clearwater Lake (47°22′N, 93°25′W), Crooked Lake (47°23′N, 93°17′W), Leech Lake (47°04′N, 94°33′W), Purvis Lake (47°49′N, 92°01′W), Wabana Creek (47°19′N, 93°25′W), and Wabana Lake (47°21′N, 93°28′W). Québec data were collected in Fraser Lake (45°23′N, 72°10′W), lac Bonny (46°05′N, 74°05′W), lac Brôme (45°18′N, 72°30′W), lac Brompton (45°25′N, 72°10′W), Lake Champlain (45°04′N, 73°08′W), lac d'Argent (45°18′N, 72°19′W), lac de l'Achigan (45°57′N, 73°58′W), lac Hertel (45°33′N, 73°08′W), lac Magog (45°18′N, 72°03′W), and Lake Memphrémagog (45°N, 72°14′W). Data on the Mississippi River are from Johnson (1976), Lake St. Clair from Hiltunen (1971), and Lake Biwa from Mori (1976) and Higashi and Hayashi (1964). n is the number of replicate samples included in the mean, m is the arithmetic mean number of organisms per sample, s^2 is the $n - 1$ weighted variance of m, and $\chi^2 = s^2 (n - 1)/m$ (Elliott 1977). Asterisks indicate significant departures from a random spatial distribution: $*p < 0.05$; $**p < 0.01$; $***p < 0.001$; NS indicates that no significant departure from $m = s^2$ was found. Taxa indicated as "mixed species" contained primarily *Elliptio complanata* but a few *Anodonta grandis*.

Species	Water body	Spatial density (no.·m^{-2})	Quadrat size (cm^2)	n	m	s^2	χ^2
Amblema costata	Mississippi River	2.8	1 800	4	0.50	1.00	6.00 NS
Anodonta grandis	Clearwater Lake	0.6	10 000	25	0.60	1.50	60.00***
Anodonta grandis	Crooked Lake	3.4	10 000	25	3.36	8.24	58.86***
Anodonta grandis	Lac Hertel	0.1	10 000	27	0.15	0.13	23.01 NS
Anodonta grandis	Lac d'Argent	0.4	10 000	11	0.36	0.45	12.63 NS
Anodonta grandis	Purvis Lake	0.9	10 000	15	0.87	0.70	11.23 NS
Anodonta grandis	Wabana Lake	0.2	10 000	21	0.19	0.16	17.00 NS
Anodonta grandis	Wabana Lake	0.6	10 000	26	0.58	0.49	21.40 NS
Anodonta grandis	Wabana Lake	0.8	10 000	26	0.85	1.90	56.00***
Anodonta grandis	Wabana Lake	1.6	10 000	25	1.56	3.17	48.82**
Elliptio complanata	Lac Brôme	32.0	1 000	5	3.20	5.20	6.50 NS
Elliptio complanata	Lac Brôme	89.0	1 000	10	8.90	18.54	18.75*
Elliptio complanata	Lac Brompton	15.0	1 000	10	1.50	19.39	116.33***
Elliptio complanata	Fraser Lake	6.1	10 000	33	6.12	6.73	35.21 NS
Elliptio complanata	Lac Hertel	4.8	10 000	27	4.81	5.23	28.29 NS
Elliptio complanata	Lac de l'Achigan	3.3	10 000	7	3.29	3.91	7.13 NS
Elliptio complanata	Lac de l'Achigan	18.1	10 000	7	18.14	44.14	14.60*
Elliptio complanata	Lac Bonny	21.0	10 000	15	21.00	55.57	37.05***
Elliptio complanata	Lac d'Argent	19.6	10 000	11	19.55	108.07	55.28***
Elliptio complanata	Lac de l'Achigan	1.4	10 000	7	1.43	0.95	4.00 NS
Elliptio complanata	Lac de l'Achigan	6.0	10 000	7	6.00	2.67	2.67 NS
Elliptio complanata	Lac de l'Achigan	6.0	10 000	7	6.00	7.00	7.00 NS
Elliptio complanata	Lac de l'Achigan	8.7	10 000	32	8.70	16.79	59.82***
Elliptio complanata	Lac de l'Achigan	9.6	10 000	7	9.57	24.29	15.22*
Elliptio complanata	Lac de l'Achigan	11.4	316	36	0.36	0.41	39.62 NS
Elliptio complanata	Lac de l'Achigan	13.7	10 000	7	13.71	14.57	6.38 NS
Elliptio complanata	Lac de l'Achigan	18.7	10 000	12	18.67	42.42	25.00**
Elliptio complanata	Lac de l'Achigan	24.2	10 000	12	24.17	115.06	52.37***
Elliptio complanata	Lac de l'Achigan	24.2	1 000	36	2.42	5.16	74.79***
Elliptio complanata	Lac de l'Achigan	26.6	10 000	36	26.56	150.20	197.96***
Elliptio complanata	Lac de l'Achigan	27.0	3 162	36	8.53	20.48	84.07***
Elliptio complanata	Lac de l'Achigan	27.8	100	36	0.28	0.21	26.00 NS
Elliptio complanata	Lake Champlain	39.7	10 000	3	39.67	81.33	4.10 NS
Elliptio complanata	Lake Memphrémagog	5.0	1 000	10	0.50	0.50	9.00 NS
Elliptio complanata	Lake Memphrémagog	5.0	1 000	10	0.50	0.94	17.00*
Elliptio complanata	Lake Memphrémagog	11.0	1 000	10	1.10	0.99	8.09 NS
Elliptio complanata	Lake Memphrémagog	42.0	1 000	15	4.20	9.17	30.57**
Elliptio complanata	Lake Memphrémagog	47.6	100	21	0.48	0.46	19.40 NS
Elliptio complanata	Lake Memphrémagog	53.0	10 000	8	53.00	549.71	72.60***
Elliptio complanata	Lake Memphrémagog	54.5	316	18	1.72	1.15	11.39 NS
Elliptio complanata	Lake Memphrémagog	56.5	3 162	14	17.86	12.75	9.28 NS
Lampsilis radiata	Clearwater Creek	3.3	10 000	3	3.33	4.33	2.60 NS
Lampsilis radiata	Clearwater Lake	0.8	10 000	25	0.84	1.22	34.94 NS
Lampsilis radiata	Crooked Lake	0.7	10 000	25	0.68	1.14	40.35*
Lampsilis radiata	Crooked Lake	0.8	10 000	25	0.80	3.00	90.00***
Lampsilis radiata	Crooked Lake	14.3	10 000	25	14.32	67.14	112.53***
Lampsilis radiata	Lake Champlain	29.3	10 000	3	29.33	158.33	10.80***
Lampsilis radiata	Leech Lake	0.1	10 000	22	0.09	0.09	20.00 NS
Lampsilis radiata	Wabana Creek	0.4	10 000	25	0.40	0.83	49.98**
Lampsilis radiata	Wabana Lake	0.4	10 000	26	0.38	0.25	16.00 NS
Lampsilis radiata	Wabana Lake	0.8	10 000	26	0.85	1.58	46.55**
Lampsilis radiata	Wabana Lake	1.2	10 000	33	1.24	1.63	41.90 NS
Lampsilis radiata	Wabana Lake	1.7	10 000	25	1.68	1.48	21.10 NS
Lampsilis radiata	Wabana Lake	1.9	10 000	21	1.86	1.13	12.15 NS
Lampsilis radiata	Wabana Lake	3.2	10 000	20	3.15	2.24	13.51 NS
Lampsilis radiata	Wabana Lake	3.9	10 000	30	3.87	4.53	34.00 NS

TABLE 1. (*Concluded*)

Species	Water body	Spatial density (no.·m^{-2})	Quadrat size (cm^2)	n	m	s^2	χ^2
Lampsilis siliquoidea	Lake St. Clair	9.0	741	3	0.67	1.33	4.00 NS
Lampsilis siliquoidea	Lake St. Clair	27.0	741	3	2.00	11.98	11.98**
Lampsilis ventricosa	Crooked Lake	0.6	10 000	25	0.64	1.07	40.25*
Leptodea fragilis	Mississippi River	4.2	1 800	4	0.75	0.92	3.67 NS
Leptodea fragilis	Wabana Creek	0.1	10 000	25	0.12	0.36	72.00***
Ligumia sp.	Crooked Lake	2.5	10 000	25	2.52	5.18	49.30**
Mixed species	Lac Magog	5.0	1 000	10	0.50	0.50	9.00 NS
Mixed species	Lake Memphrémagog	4.0	1 000	10	0.40	4.89	110.00***
Mixed species	Lake Memphrémagog	21.1	1 000	9	2.11	4.86	18.42***
Mixed species	Lake Memphrémagog	22.2	1 000	9	2.22	2.94	10.60 NS
Mixed species	Lake Memphrémagog	30.0	100	10	0.30	2.33	70.00***
Mixed species	Lake Memphrémagog	30.7	10 000	6	30.67	141.87	23.13***
Mixed species	Lake Memphrémagog	36.0	10 000	5	36.00	226.50	25.17***
Obliquaria reflexa	Mississippi River	7.8	1 800	5	1.40	2.30	6.57 NS
Obliquaria reflexa	Mississippi River	9.7	1 800	4	1.75	2.25	3.86 NS
Truncilla donaciformis	Mississippi River	4.4	1 800	5	0.80	0.70	3.50 NS
Truncilla donaciformis	Mississippi River	11.1	1 800	4	2.00	3.33	5.00 NS
Unio biwae	Lake Biwa	44.0	225	3	0.99	0.98	1.98 NS
Unio biwae	Lake Biwa	118.0	225	3	2.66	0.33	0.25 NS
Villosa fabilis	Lake St. Clair	9.0	741	3	0.67	0.33	1.00 NS

putational simplicity. Scheffé (1959) and others have suggested that, among the assumptions of analyses based on the normal distribution, equality of variances is the most critical. Because the variances associated with the different means were expected to be unequal (eq. 1), the variance of the difference in means ($s^{2\prime}_{m_1-m_2}$) was calculated as (according to Steel and Torrie 1980)

$$(5)\quad s^{2\prime}_{m_1-m_2} = \frac{s_1^2 + s_2^2}{n}$$

and the degrees of freedom (df′) were approximated as

$$(6)\quad \mathrm{df}' = \frac{((s^2_{\ 1} + s_2^2)/n)^2}{((s_1^2/n)^2 + (s_2^2/n)^2)/(n-1)}$$

where s_1^2 and s_2^2 were estimated from eq. 1 and n is the number of samples used in the estimation of both m_1 and m_2. This analysis was repeated for a variety of combinations of m_1 and m_2 at $\alpha = \beta = 0.05$. α and β were set equal to each other because in impact studies, it may be just as important to minimize the probability of failing to infer a significant difference when it actually exists (type II error) as it is to minimize the probability of inferring a significant difference when there is not one (type I error) (Parkhurst 1990; Peterman 1990).

Results and Discussion

Spatial Aggregation

Unionid mollusc populations were frequently aggregated in space, even though samples were taken within relatively homogeneous sampling strata. Of 76 comparisons, 45% showed significant spatial aggregation (Table 1). Surprisingly, however, 55% of the s^2/m could not be discerned from a Poisson distribution. Unionid mussel populations had aggregated distributions much less frequently than other benthic taxa. Figure 1, however, shows that populations sampled with $n < 9$ and $m < 10$ yielded s^2/m that indicated no significant aggregation more frequently than populations sampled with

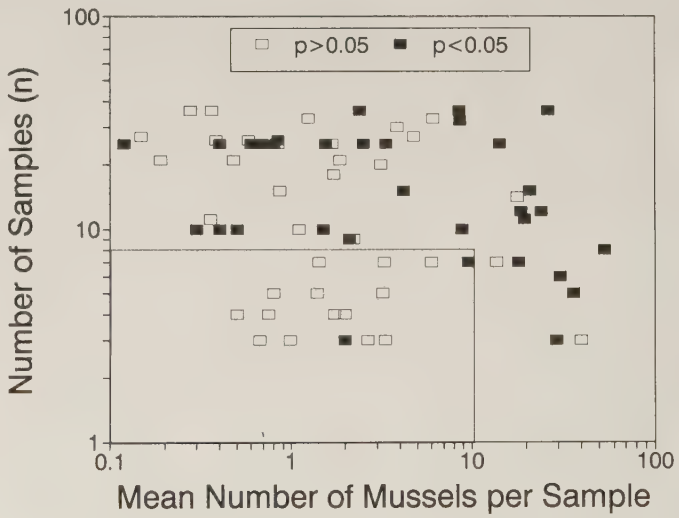

FIG. 1. Results of chi-square tests of s^2/m to test for significant spatial aggregation in populations of unionid mussels. Open squares indicate that no significant aggregation was seen, and closed squares indicate that chi-square values were statistically significant ($p < 0.05$). The rectangle encloses the region where $n < 9$ and $m < 10$.

more sampling units and in which larger numbers of mussels were collected. Our data suggest that either aggregation is rare or chi-square tests are unlikely to detect spatial aggregation at low n and m. Even ignoring populations with $n < 9$ and $m < 10$, however, we found that only 53% of mussel populations were significantly aggregated. The data with $n \geq 9$ and $m > 10$ suggested that mussel populations occurring at higher spatial densities were more frequently aggregated (up to 86% of populations) than those at lower densities (Fig. 2). Elliott's (1977) assertion that "The spatial dispersion of a population is seldom random or regular. . ." does not seem to apply to unionid molluscs. Many freshwater mussel populations are not significantly aggregated within their habitat.

The sampling variance obtained for unionid populations varied as a significant function of the average number of organisms collected:

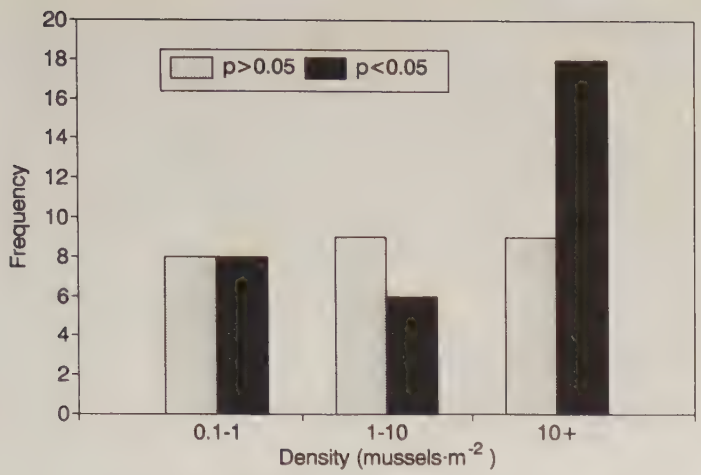

FIG. 2. Frequency of significant aggregation (chi-square test, $p < 0.05$) in populations of unionid mussels found at different spatial densities. Only populations sampled with at least $n = 9$ and $m = 10$ are included.

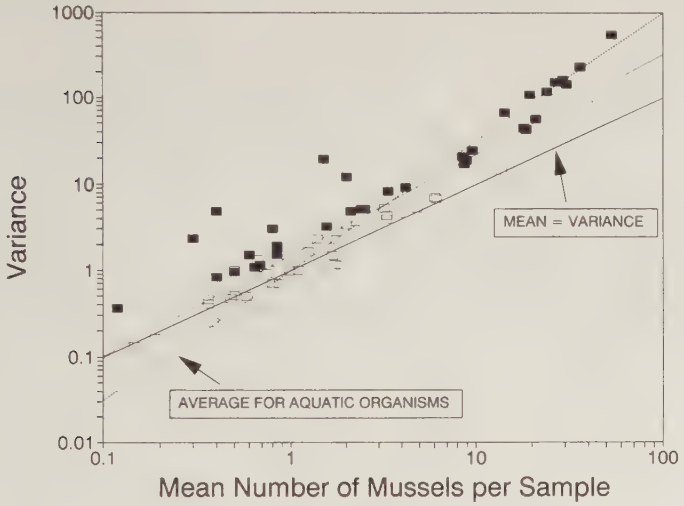

FIG. 3. Relationship between the variance (s^2) and mean (m) of replicate samples of freshwater mussel populations. The solid line indicates $s^2 = m$ (randomness) and the dotted line indicates $s^2 = m^{1.5}$ (eq. 2). Closed and open squares indicate that the chi-square test (Elliott 1977) for agreement with the Poisson distribution showed significant ($p < 0.05$) or lack of significant difference ($p > 0.05$), respectively.

(7) $s^2 = 1.49m^{1.168}$

(log–log regression: $n = 76$, $r^2 = 0.85$, RMS = 0.1121, $F = 413$, $p << 0.0001$). This relationship is illustrated in Fig. 3. Only 3 of the 28 such relationships for variance of freshwater organisms reviewed by Downing (1991) had lower exponents (Fig. 4), but this is probably due to the larger average number of replicate samples included in m and s^2 calculations for unionid molluscs. Downing (1986) has shown that these empirically derived exponents (b in eq. 1) vary approximately as $b = 2.057 - 0.11 \log_{10} n_v - 0.37 \log_{10} n_r$, where n_v is the number of replicate population estimates included in m and s^2, and n_r is the number of m:s^2 pairs included in each regression of s^2 on m. Given the average n_v of 16 (Table 1) and the 76 points included in the derivation of eq. 7, we would expect a priori an exponent of about 1.23. The low exponent probably results, in part, from the sampling designs used for unionid mussels.

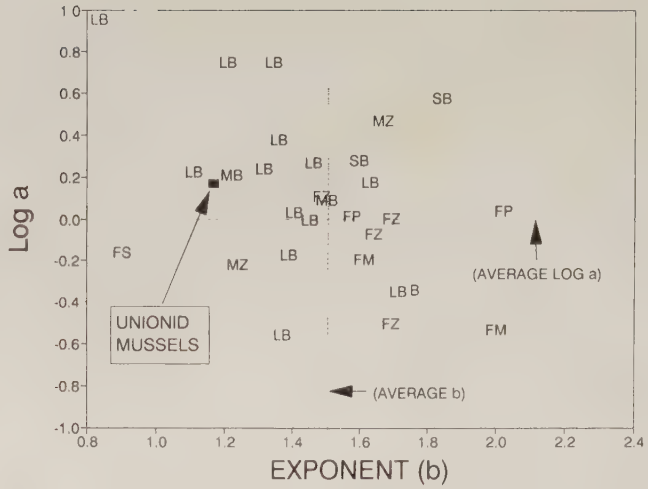

FIG. 4. Coefficients of the relationship between m and s^2 in published studies reviewed by Downing (1991). All m are expressed on a per sample basis, not normalized to common units of volume or surface area. Abbreviations for organism type: LB, lake benthos; SB, stream benthos; MB, marine benthos; FZ, freshwater zooplankton; MZ, marine zooplankton; FP, freshwater phytoplankton; B, bacteria; FS, freshwater sediment; FM, freshwater macrophytes. Literature sources are listed in Downing (1991).

Unionid mussel spatial distributions seem to approach randomness (i.e. $m = s^2$) when few organisms were collected in samples but follow the average relationship between m and s^2 found for other aquatic organisms (eq. 2) when many mussels were collected (Fig. 3). The variances of mussel samples tend toward the $s^2 = m$ line, indicating randomness at $m < 1$, but mussel variances depart from a $s^2 = m$ trend and follow eq. 2 more closely when $m > 1$. Apparent randomness at low m is a sampling artifact that arises because the minimum and maximum possible variances for population count data converge on $m = s^2 = 1/n$ at low m (Downing 1989, 1991). Sets of samples with $m < 1$ must approach randomness by mathematical necessity, and thus, their capacity to infer spatial pattern is greatly impaired. This also explains the low power of chi-square tests for spatial aggregation at low n and m (Fig. 1 and 2). For $m > 1$, ANOVA of the residuals of the data in Table 1 from eq. 2 shows that the degree of spatial heterogeneity of unionid mussel populations does not depart significantly from that seen in many other aquatic taxa ($p = 0.18$); thus, $s^2 = m^{1.5}$ may be considered a general rule followed closely by unionid mussels.

Prediction of Sampling Requirements

Equation 2 allows provisional estimates of requisite sample number for unionid mussels and other taxa to be made. The number of requisite samples for a specified degree of precision ($D = \text{SE}/m$) of mean densities of $m \geq 1$ can be estimated by substituting the coefficients of eq. 2 into eq. 3:

(8) $\hat{n} = 1 \cdot m^{-0.5} D^{-2}$.

Calculations of the number of required replicate samples for various spatial densities (number per square metre as opposed to number per sample) can be made by substituting [(number per square metre)/(10 000/A)] for m in eq. 8, where A is the area (square centimetres) covered by each replicate sample. Equation 7 is not used because it is biased by artifactually low s^2 at $m \leq 1$. Table 2 shows that many replicate samples must

TABLE 2. Predicted requisite number of samples of unionid molluscs to obtain a precision of 20% (i.e. SE/m = 0.2). Predictions of n from eq. 3 are shown for the range of spatial densities and sampler sizes encountered in the literature. Where $m < 1$, the sample numbers are the minimum number that could theoretically yield $D = 0.2$ (Downing 1989) or $[(1 - m)/(mD^2)] - 1$.

Density (no.·m^{-2})	Size of sampler (cm^2)				
	100	300	1000	3000	10 000
1	2474	807	224	57	25
3	807	252	57	26	15
10	224	57	25	15	6
30	57	26	14	8	5
100	25	14	8	5	2

be taken to obtain a precision of SE/m = 0.2. As with most other faunae, Table 2 shows that the number of replicate samples needed decreases with increased spatial density and sampler size. Even when very large numbers of mussels are collected in samples, however, precise population estimates usually require more than 10 replicate samples to be taken. This is especially sobering considering that the most frequent number of benthos samples taken by aquatic ecologists is three (Downing 1979). In fact, more than 85% of published estimates of mean density of benthic invertebrates are based on three samples or less (J. A. Downing, unpubl. obs.). In contrast with other benthic faunae, most of the cost of unionid mollusc population estimation is probably associated with the taking of the samples themselves (they are easy to separate from sediment and count); therefore, larger sampling units are probably best for sampling freshwater mussels (Table 2). This stategy will ensure large m and acceptable sampling precision, but will also permit robust chi-square tests for spatial aggregation.

Although our analysis is based on limited data, we believe that it is representative of samples that will be encountered in the field. Our data bracket the range of habitats and densities in which unionids are found (0.1–118·m^{-2}; Table 1). For example, Coker et al. (1922) examined many populations of different species of freshwater mussels and indicated that the densest populations had 31 organisms·m^{-2}. These authors cited others who found densities as high as 178·m^{-2}, suggesting that any spatial density >43·m^{-2} is "unusual." Negus (1966) found mean densities as high as 42·m^{-2}, Cvancara (1970) as high as 54·m^{-2}, and Green (1980) as high as 36·m^{-2}. Small exotic bivalves like the Asian Clam, however, have been observed at densities up to 1475·m^{-2} (Miller et al. 1986), and zebra mussel densities may be even higher. The lack of significant departure of our data on s^2 and m from a general relationship based on more than 18 000 sets of samples (eq. 2) lends support to the generality of this m:s^2 rule and suggests that our findings may be extrapolated to bracket the high densities observed in exotic mussels. Because spatial heterogeneity may vary somewhat among species or in extreme habitats (e.g. Downing 1979), however, it would be prudent to temper predictions from eq. 8 with site-specific sampling experience.

Detecting Differences among Population Means

Many future surveys of unionid mussel abundance will be performed to detect the influence of exotic species or pollution on mussel communities. In such cases, it is not simply the precision associated with each population estimate that is important (e.g. Downing 1979; Morin 1985; Vézina 1988; Riddle 1989), but rather the ability to discern or detect changes in population levels, before and after changes in the environment. We can use our analysis of mussel spatial variation to determine the number of samples that must be taken to differentiate between two hypothetical mean densities.

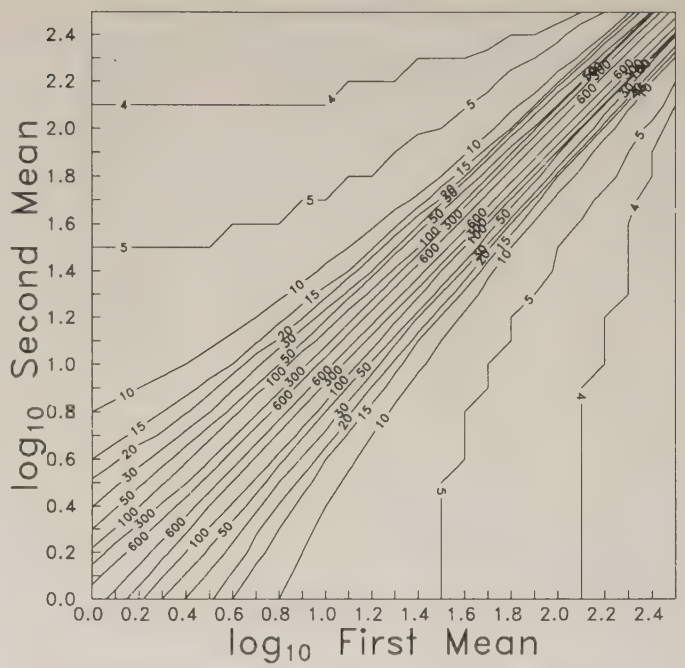

FIG. 5. Minimum number of samples required to estimate each mean mussel density in order to find a significant difference ($\alpha = \beta = 0.05$) between two freshwater mussel densities ($\log_{10}$). Calculations were made by substituting eq. 2 into eq. 4–6 and iterating n until $\alpha = \beta = 0.05$ (Cyr et al. 1992).

The number of replicate samples needed to detect significant differences decreases as the means and the differences among means increase (Fig. 5). Sampling surveys based on the median number of replicate samples taken in benthos studies (n = 3) could not even detect significant ($\alpha = \beta = 0.05$) differences of two orders of magnitude. Detection of doubling or halving of the population density would take about 50 samples to derive each mean at m = 1, around 15–20 samples at m = 10, and about five samples at m = 100. Even the detection of order-of-magnitude changes in population density would require between three and nine samples for the derivation of each of the estimated means involved in the comparison. Again, the use of large sampling units is indicated because mussel densities are rarely >100·m^{-2} and will decline when impacted, requiring greater numbers of samples. Our calculations show that most sampling designs currently used for sampling benthic organisms are inadequate for detecting changes in the abundance of unionid mussels.

This research has shown that unionid molluscs are frequently aggregated in space. A sufficient fraction of this spatial variability can be predicted based on estimates of the spatial density so that sampling programs can be planned without population-specific knowledge of the spatial pattern. Current approaches to sampling must be revised radically if we wish to monitor changes in unionid communities faced with pollution and introduced exotic species.

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Phytofauna of Eleven Macrophyte Beds of Differing Trophic Status, Depth, and Composition[1]

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Lalonde, S., and J. A. Downing. 1992. Phytofauna of eleven macrophyte beds of differing trophic status, depth, and composition. Can. J. Fish. Aquat. Sci. 49: 992–1000.

Macrophyte beds in 11 lakes of differing trophic conditions were sampled intensively to examine the influence of macrophyte abundance and composition, epiphyton biomass, phytoplankton concentration, and water depth on the abundance of phytophilous invertebrates. Numerical abundance and biomass of phytofaunal taxa were only weakly correlated. Phytofauna biomass ranged from 17 to 270 mg dry mass·g macrophyte dry mass^{-1} (1–29 g dry mass·m^{-2}) among the macrophyte beds. Multiple regression analysis showed that total phytofaunal biomass was positively correlated with the biomass of the three primary producers in the littoral zone: macrophytes, epiphyton, and phytoplankton. Phytofauna biomasses in deeper macrophyte beds or near the water surface were lower than those found in shallower water or near the sediment surface. Correlations of phytofauna biomass with macrophytes, epiphyton, and depth varied somewhat among phytofaunal taxa. The phytofauna biomass was often dominated by chironomid larvae, but gastropods, water mites, and oligochaetes were also important components of the phytofauna biomass. Small crustaceans such as cladocerans and copepods frequently were numerically dominant but usually composed only a small fraction of the biomass. Preference of various invertebrate taxonomic groups for particular species of aquatic macrophyte was slight.

Onze herbiers de macrophytes situés dans des lacs différents furent échantillonnés afin d'étudier l'influence de l'abondance et de la composition en macrophytes, la biomasse d'épiphyton, la concentration de phytoplancton et la profondeur de l'eau sur l'abondance des invertébrés phytophiles. Bien que la plupart des études antérieures portant sur la phytofaune analyse l'abondance relative des invertébrés en fonction des nombres d'organismes, nous avons trouvé que l'abondance numérique et la biomasse des divers taxa sont faiblement corrélées. La biomasse de phytofaune variait beaucoup entre les herbiers allant de 17 à 270 mg masse sèche·g masse sèche de macrophyte^{-1} et représentant de 1 à 29 g masse sèche·m^{-2}. Une analyse de régression multiple a démontré que la biomasse totale de phytofaune était corrélée de façon positive avec la biomasse des trois producteurs primaires de la zone littorale : les macrophytes, l'épiphyton et le phytoplancton. Dans les herbiers les plus creux ou près de la surface de l'eau, les biomasses de phytofaune étaient plus faibles que dans les eaux peu profondes et près des sédiments. Les corrélations entre la biomasse de phytofaune et les macrophytes, l'épiphyton et la profondeur étaient variables entre les divers taxa d'invertébrés. La biomasse de phytofaune était fréquemment dominée par les larves de chironomides mais les gastéropodes, les acariens et les oligochètes constituaient aussi d'importants représentants de la biomasse de phytofaune. Les petits crustacés tels les copépodes et les cladocères, bien que fréquemment dominants en nombres, constituaient généralement une petite fraction de la biomasse. La préférence de certains groupes taxonomiques d'invertébrés pour des espèces particulières de macrophytes aquatiques était faible.

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Invertebrates colonizing the surfaces of aquatic macrophytes (phytofauna) are very abundant and can contribute a large fraction of the secondary production in lakes (Straškraba 1965; Pieczyński 1973; Lim and Fernando 1978; Schramm and Jirka 1989). They are important food for fish and waterfowl (Krull 1970; Danell and Sjöberg 1980; Kaminski and Prince 1981; Fairchild 1982; Keast 1984; Schramm and Jirka 1989). The phytofauna is a strategic link in the cycling of energy and nutrients in aquatic ecosystems (Jónasson 1978; Miura et al. 1978; Kołodziejczyk 1984b; Kairesalo and Koskimies 1987). Unfortunately, the technical difficulty and expense of sampling this important fauna have impeded study of it (Downing 1984). Most studies of the phytofauna have been based upon qualitative or inherently biased sampling methods (see Downing and Cyr 1985), and few have attempted to make comparisons among the phytofauna found in more than a few ecosystems.

Until recently, studies of invertebrate:macrophyte relationships have focused primarily on the preference of various invertebrate species for certain macrophyte species (e.g. Krecker 1939; Andrews and Hasler 1943; Rosine 1955; Soszka 1975a; Pip and Stewart 1976; Biggs and Malthus 1982; Talbot and Ward 1987) or the direct use of macrophytes by the phytofauna (e.g. McGaha 1952; Gaevskaya 1966; Soszka 1975b). The importance of the phytofauna as food for other trophic levels, however, suggests the importance of knowing which characteristics of lakes and macrophyte beds influence the development of phytofaunal biomass.

A few recent studies have attempted to quantify the influence of some environmental characteristics on the biomass of phytophilous invertebrates in macrophyte beds. Some (e.g. Vincent et al. 1982; Cyr and Downing 1988a) have investigated

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the influence of macrophyte species composition, sediments, current speed, or other environmental characteristics on the numerical abundance of the phytofauna. Although the numerical abundance is useful in discovering factors influencing specific phytofaunal taxa, biomass estimates would yield information more relevant to the study of energy transfers in lacustrine ecosystems. Rasmussen (1988) developed several models predicting the biomass of littoral zoobenthos based on the littoral slope, wave exposure, sediment characteristics, trophic status, and the chemical composition of the water. This important study treats only the aggregate of the littoral benthos (sediment and plant-dwelling animals) and thus furnishes no information specific to the phytofauna, nor does it allow predictions of the biomass of groups of organisms that are of particular importance to the nutrition of littoral fish and waterfowl (e.g. Gascon and Leggett 1977; Mittelbach 1984; Keast 1985).

The phytofauna biomass found in a given ecosystem could be influenced by several factors. Several authors have suggested that the abundance of littoral invertebrates is correlated with the biomass and species composition of their macrophytic substrate (Vincent et al. 1982; Downing 1986; Cyr and Downing 1988a). High standing macrophyte biomass may decrease wave-induced turbulence which could detach epiphytic animals (Bownik 1970), thus favoring high phytofauna biomass. Epiphytic algae are an important food source for invertebrates (Mason and Bryant 1975; Cattaneo and Kalff 1980), and epiphyton-grazing invertebrates constitute a large proportion of the phytofauna. Several authors, therefore, have suggested that epiphytes are important to phytofaunal production (Petersen and Boysen-Jensen 1911; Entz 1947; Rosine 1955; Gaevskaya 1958; Harrod 1964; Foerster and Schlichting 1965; Soszka 1975b; Schramm et al. 1987), although few explicit tests exist (Downing 1981; Fairchild 1981; Cattaneo 1983; Ohtaka and Morino 1986). Because many phytofaunal organisms can also filter-feed (Downing 1981; Iversen et al. 1985; Harper 1986), phytoplankton biomass may have a positive influence on phytofaunal biomass. We might thus expect higher phytofaunal biomasses in eutrophic lakes (Lemly and Dimmick 1982; Talbot and Ward 1987). Because shallow macrophyte beds receive greater solar radiation and are closer to sediment nutrients, lower invertebrate biomasses may be found in deeper macrophyte beds (Soska 1975a). On the other hand, animals found on macrophyte stems nearest the water's surface might be most frequently detached by wave action (Entz 1947; Pieczyński 1964; Bownik 1970).

The objective of this study was to find whether the phytofauna biomass in diverse macrophyte beds in several dissimilar lakes is related to the biomass and composition of macrophytes, the biomass of epiphytic and phytoplanktonic algae, the mean depth of the macrophyte bed, as well as the relative proximity of the fauna to the sediment and lake surface.

Methods

This study was performed in 11 lakes in southern Québec (Fig. 1) during July and August 1985 and July 1986. Sampling sites covered a wide range of density and composition of macrophytes, epiphyton biomass, and lake trophic status. Data collection began in July, shortly after macrophyte biomass was well established and the macrophytes had been well colonized by invertebrates. One macrophyte bed was sampled once in each lake. The 11 macrophyte beds were sampled in random order. The biomass of the phytofauna was estimated at each site and the relationship between these standing biomasses and environmental characteristics was determined using regression analysis. Macrophyte, epiphyton, and phytoplankton biomass and depth were considered independent variables in these regression analyses.

The phytofauna was sampled following the protocol of Downing (1986). Divers collected animals by gently closing a water-tight Plexiglas box (6 L) around the macrophyte stems and leaves without disrupting the epiphytic flora or fauna. Twelve samples (six in lac Fournelle) were taken at randomly chosen points along a 50-m transect parallel to shore at each sampling site. We sampled near the water surface, middepth, and near bottom, and the distance between the box-sampler and the sediment surface (Z_b, metres) was noted for each sample (Z_b ranged from 0.1 to 2.5 m). The average depth of the macrophyte bed along the transect ($\bar{Z}$, metres; mean of six measurements) was determined at each site.

The volume of each 6-L box-sample was reduced in the field by passing it through a filter-funnel (Nytex, 100 μm; Likens and Gilbert 1970). Samples were kept cold during transport to the laboratory where the phytofauna was separated from the macrophytes by rinsing the plant surfaces with a gentle jet of filtered water over a 100-μm sieve. Rinsed pieces of macrophytes were examined regularly to make certain that animals were removed quantitatively. Invertebrates were preserved in 80% ethanol with 1% glycerine added to avoid desiccation. The macrophyte pieces collected in these samples were identified following Fassett (1957), rinsed again, dried (60°C), and weighed (± 0.1 mg).

All of the invertebrates found in our 126 samples were identified and counted using a dissecting microscope (16×). Detailed taxonomic analysis was not performed, but invertebrates were classified into eight major groups: Copepoda, Cladocera, Chironomidea, Trichoptera, Oligochaeta, Acari, Ostracoda, and Gastropoda. Biomasses of these groups were determined by measuring the lengths of individuals of different taxa in a subsample of each of the 126 samples. The frequency distribution of body length for each taxon was determined for each sampling site. This length–frequency distribution was converted to a mass–frequency distribution using published length–mass relationships (e.g. Dumont et al. 1975; Bottrell et al. 1976; Smock 1980; Rosen 1981; see Lalonde 1988). Gastropod biomasses excluded the shell.

After each box-sample was sealed and brought to the boat, a subsample of macrophyte and its epiphytes was withdrawn through an opening in the side of the sampler. The epiphyton biomass (EPI, micrograms chlorophyll *a* per gram dry mass of macrophyte) was determined using Cattaneo and Kalff's (1978) method. Each small macrophyte subsample was transferred gently to a clean jar containing 200 mL of filtered lake water, and the epiphytes were dislodged from the macrophytes by vigorous shaking. A subsample of 20–200 mL of epiphyte suspension was filtered (Millipore prefilters) and chlorophyll *a* was later extracted by soaking filters in 96% ethanol for 24 h in the dark under refrigeration. The chlorophyll solution was filtered to reduce turbidity and read spectrophotometrically at 665 and 649 nm (Bergman and Peters 1980). The macrophytes from which epiphyton was removed were identified, dried, and weighed as above.

The chlorophyll concentration of the lake water around the macrophytes (S, micrograms chlorophyll *a* per litre) was estimated at 18 random points along the transect at each site. Water

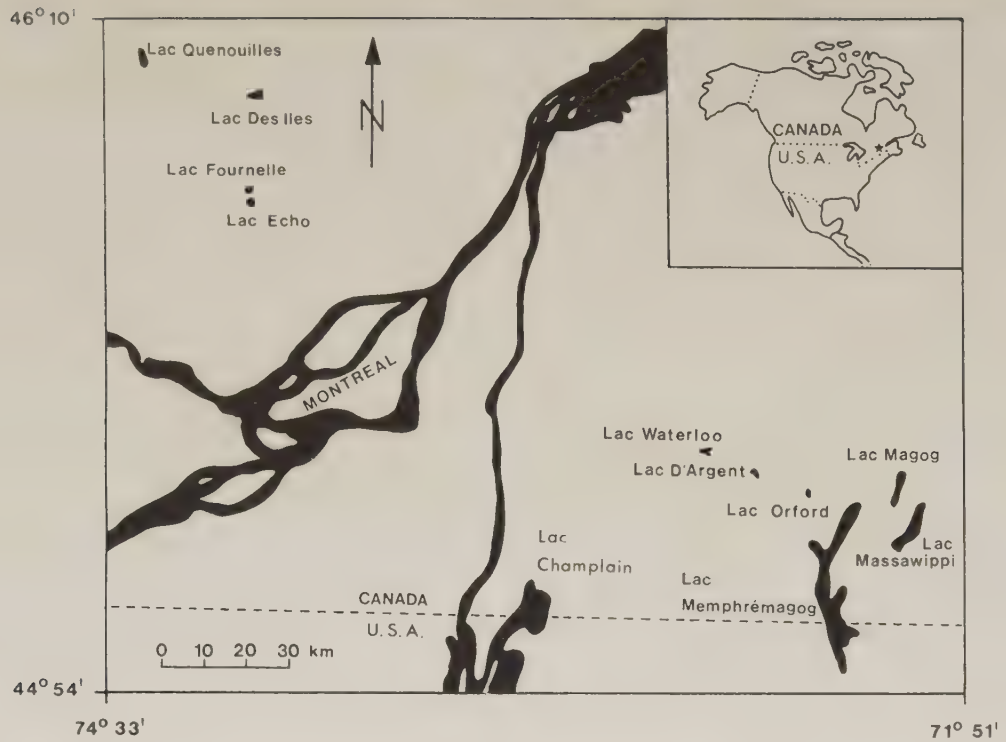

FIG. 1. Map of southern Québec showing the location of the 11 lakes in which macrophyte beds were sampled.

TABLE 1. Characteristics of the 11 macrophyte beds sampled. AMB is the mean areal macrophyte biomass (g macrophyte dry mass·unit lake bottom $^{-1}$); TP is the mean total phosphorus concentration of the water column ($\mu g \cdot L^{-1}$); S is the average phytoplankton biomass within the macrophyte bed (μg chlorophyll $a \cdot L^{-1}$); EPI is the mean epiphyton biomass (μg chlorophyll $a \cdot g$ macrophyte dry mass^{-1}); and $\bar{Z}$ is the mean depth of the sampled site (m). Phytofauna biomass is expressed per unit of macrophyte biomass (mg dry mass·g dry mass^{-1}) and per unit of lake bottom ($g \cdot m^{-2}$). Standard deviations are in parentheses. The species of macrophytes that represented >1% of the total areal biomass are listed by decreasing order of relative importance. Cd, *Ceratophyllum demersum*; Ch, *Chara* sp.; Cla, *Cladophora* sp.; Ec, *Elodea canadensis*; Hd, *Heteranthera dubia*; J, *Juncus* sp.; M, *Myriophyllum spicatum* (except for lac des Iles, where we found *M. humile*); N, *Nitella* sp.; Pa, *Potamogeton amplifolius*; Pr, *Potamogeton robbinsii*; Pri, *Potamogeton richardsonii*; Pp, *Potamogeton praelongus*; P, *Potamogeton* sp. (similar to *P. epihydrus* in form); S, *Sagittaria* sp.; Va, *Vallisneria americana*. Taxonomy followed Fassett (1957). Sample sizes (n): AMB = 17–25, TP = 4–6, S = 13–18, EPI = 20–32, $\bar{Z}$ = 6, and phytofauna biomass = 12 (6 in Lake Fournelle).

Macrophyte bed	AMB ($g \cdot m^{-2}$)	TP ($\mu g \cdot L^{-1}$)	S ($\mu g \cdot L^{-1}$)	EPI ($\mu g \cdot g^{-1}$)	$\bar{Z}$ (m)	Phytofauna		Dominant macrophytes
						$mg \cdot g^{-1}$	$g \cdot m^{-2}$	
des Iles	36	6	2	480	1.6	74	3	M, Pa, Pr,
	(35)	(1)	(1)	(616)	(0.1)	(10)	(4)	Ni, P
Orford	61	7	2	235	2.0	36	2	Pa, Ec, Ni
	(34)	(2)	(1)	(196)	(0.3)	(45)	(3)	
Fournelle	261	7	3	466	1.6	47	12	Pr, Ec
	(194)	(1)	(1)	(382)	(0.3)	(37)	(10)	
Quenouilles	39	11	2	217	1.3	17	1	Pa, J, Ch, S,
	(18)	(3)	(1)	(219)	(0.1)	(11)	(1)	Va
Echo	521	13	8	142	1.6	33	17	Ch, Pa, Pr,
	(375)	(6)	(8)	(127)	(0.1)	(13)	(7)	Pp
d'Argent	34	13	8	209	1.5	80	3	M, Ni, Va
	(25)	(3)	(2)	(164)	(0.3)	(29)	(1)	
Memphrémagog	70	18	3	114	2.2	60	4	M, Va, Hd,
(Cove Island Bay)	(51)	(4)	(1)	(83)	(0.1)	(30)	(2)	Ec
Champlain	112	23	4	120	2.0	24	3	Va, Cla, Pri
(Bay of Venise)	(60)	(4)	(2)	(89)	(0.0)	(12)	(1)	
Massawippi	215	24	16	995	2.2	22	5	M
	(121)	(9)	(12)	(484)	(0.4)	(10)	(2)	
Magog	149	58	20	414	1.6	192	29	Ec, M, Pr,
	(62)	(21)	(13)	(310)	(0.1)	(87)	(13)	Cd
Waterloo	105	73	42	122	1.1	270	28	M, Va
	(157)	(17)	(11)	(52)	(0.1)	(131)	(14)	

samples were taken by a diver using a 1-L opaque plastic bottle at randomly chosen depths beneath the water surface. The chlorophyll *a* concentration of the filtrate (Millipore prefilters) was determined as above, before and after acidification (Nusch 1980). The total phosphorus concentration in unfiltered lake water (TP, micrograms per litre) was determined at 6 points along the transect at each site (every 10 m). Water samples were taken ≈30 cm under the water surface. TP was measured using persulfate digestion followed by the ascorbic acid colorimetric method (American Public Health Association et al. 1985). Because variations in TP and S within macrophyte beds were very small (Table 1) (Cyr and Downing 1988a), TP and S estimates were not paired with invertebrate biomass estimates but were analyzed as macrophyte bed averages.

Average areal macrophyte biomass at each sampling site (AMB, grams per square metre) was estimated by the quantitative collection of macrophytes in 20 square quadrats (361 cm^2; Downing and Anderson 1985) placed randomly along the 50-m transect at each site. Phytofaunal biomass per unit area was estimated as the product of phytofauna biomass per gram of macrophyte dry mass and the standing macrophyte biomass (grams dry macrophyte per square metre).

We collected our samples within the shortest possible time period because we wanted to minimize temporal variability in phytofaunal biomass. Other researchers have often found the maximum phytofauna biomass during early summer. Our samples were taken during mid- to late summer, when phytofauna biomass is usually relatively stable (Soszka 1975a; Cattaneo 1983). Because the phytofauna biomass is not perfectly stable throughout this short period, however, the sampling date as elapsed time (days) was employed as an independent variable in the statistical analyses to ensure that seasonal variation would not confound our analyses. The value of this variable ranged from 1 (earliest date sampled) to 44 (latest date sampled).

The correlation of phytofauna biomass to macrophyte, epiphyte, and phytoplankton biomass, TP, and depth was tested using multiple, linear, stepwise regression analysis employing backward eliminations of insignificant variables. Regression analyses were thus performed both for the biomass of different taxa and the sum of all phytofauna biomass. Candidate variables in these analyses were the biomass of macrophyte from which invertebrates were collected (BP), EPI, phytoplankton biomass (S), TP, AMB, $\bar{Z}$, Z_b, and the sampling date. Some of these explanatory variables were estimated independently for each phytofauna sample (i.e., BP, EPI, and Z_b), while others varied little within macrophyte beds (i.e. AMB, TP, S, $\bar{Z}$, and date) and their averages within beds were used as ranked dummy variables in the multiple regression analyses. Regression analyses were thus performed following the protocol outlined by Draper and Smith (1981) for regressions using mixtures of quantitative and qualitative variables. Only variables with significant ($p < 0.05$) partial F-values were retained. Because S and TP are often highly correlated in lakes (e.g. Dillon and Rigler 1974), they are redundant measures of lake trophic status; thus, only the variable with the greatest significance was retained in regression analysis. We interpret the statistical significance of either of these variables to mean that phytofauna biomass was correlated with the abundance of suspended matter. The only other high collinearity among independent variables ($|r| > 0.5$) was a marginally significant ($p = 0.037$) negative correlation between log S and date. Gujarati (1978) suggested that collinearities with $|r| < 0.05$ pose little danger to interpretation of regression results. Because the data from the two sampling years were pooled, we have tested for year-to-year differences using an analysis of variance on the residuals of the multiple regressions.

The importance of particular macrophyte species was examined by multiple regression analysis as above, except that the macrophyte biomass per sample was partitioned into the biomasses represented by each of the 12 macrophyte species found in these macrophyte beds. The added ecological information content of the macrophyte biomass thus partitioned was judged by testing for significant increases in R^2 when considering macrophyte species biomasses (Gujarati 1978, p. 132–134). Data were transformed, where necessary, to normalize and stabilize the variance of the residuals, and the residuals of all regression analyses were examined using standard methods (Gujarati 1978).

Results and Discussion

The macrophyte beds sampled covered much of the range of ecological conditions found in littoral zones of north temperate lakes (Table 1). AMB varied between 34 and ≈500 g dry mass·m^{-2} which covers much of the range seen in natural lakes (Wetzel 1983; Duarte et al. 1986). TP and chlorophyll concentrations indicated oligotrophic to eutrophic conditions (Wetzel 1983). EPI ranged from 114 to 995 μg chlorophyll·g macrophyte biomass^{-1}. Average phytofaunal biomass also varied widely, representing as little as 17 mg dry mass·g macrophytes^{-1} to as much as 27% of standing macrophyte biomass (Table 1).

The majority of previous studies of the phytofauna have reported abundance as numbers of organisms and used these estimates to judge the relative importance of taxonomic groups to the phytofauna. We found no correlation ($r = 0.15$, $p = 0.19$) between relative numerical abundance and biomass of various taxonomic groups in these 11 macrophyte beds (Fig. 2). Ostracods and crustaceans were very important on a numerical basis but only made up a small fraction of the phytofaunal biomass. Gastropods, water mites, chironomids, and other insects, on the other hand, contributed much to the biomass but were numerically unimportant. Numerical abundance estimates therefore would not yield accurate descriptions of the community biomass composition. There was, however, a correlation between the total numbers and biomass of the total phytofauna (Fig. 3). The broad scatter around this relation indicates that the average body size of phytofaunal organisms varied widely among lakes of different trophic status. Contours of average body masses plotted in Fig. 3 show that average body sizes in eutrophic lakes such as Waterloo and Magog (macrophyte beds 10 and 11 in Fig. 3) were as much as 10 times those found in oligotrophic lakes such as lac des Iles or lac Quenouilles (macrophyte beds 1 and 4 in Fig. 3). An analysis of covariance showed that phytofaunal organisms in eutrophic lakes were significantly larger ($p < 0.0001$) than those in oligotrophic lakes.

Mean biomass of phytofauna varied widely among the 11 sampling sites (Table 1), ranging from 1 to 29 g dry mass·m^{-2}. A survey of phytofaunal biomass in other ecosystems (Fig. 4; 58 estimates expressed in comparable units, from eight literature sources) shows that the median phytofauna biomass is 1 g·m^{-2} (mean of 2.2) with extreme values ranging from 0.01 g·m^{-2} (Dvořak and Best 1982) to 12.48 g·m^{-2} (Scheffer et al. 1984). Ninety-one percent of our estimates of phytofauna

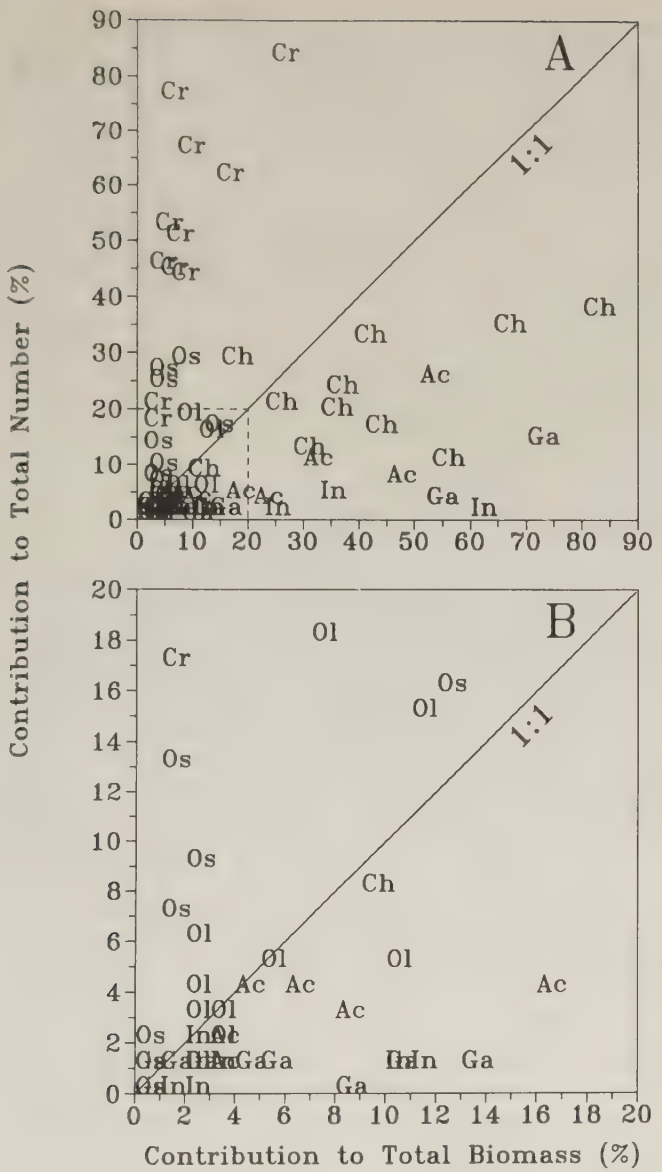

FIG. 2. (A) Relative contribution of various taxonomic groups to the total number and to the total biomass of the phytofauna (r = 0.15, p = 0.19). The line represents a 1:1 correspondance. Taxonomic groups: Ac, Acari; Ch, Chironomidae; Cr, Crustacea (Copepoda and Cladocera); Ga, Gastropoda; In, Insecta; Ol, Oligochaeta; Os, Ostracoda. Observations for each of the 11 macrophyte beds are plotted. (B) Enlargement of the lower left-hand corner of Fig. 2A.

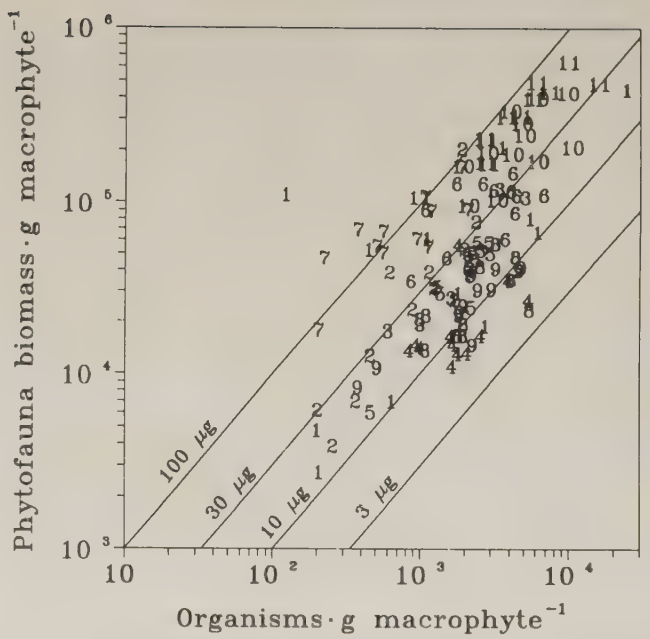

FIG. 3. Relation between the total biomass and the total numbers of all phytofaunal taxa (n = 126). All masses are as micrograms dry mass. Macrophyte beds: 1, des Iles; 2, Orford; 3, Fournelle; 4, Quenouilles; 5, Echo; 6, d'Argent; 7, Memphrémagog; 8, Champlain; 9, Massawippi; 10, Magog; 11, Waterloo. Contours indicate the relation for phytofaunal individuals of given dry mass.

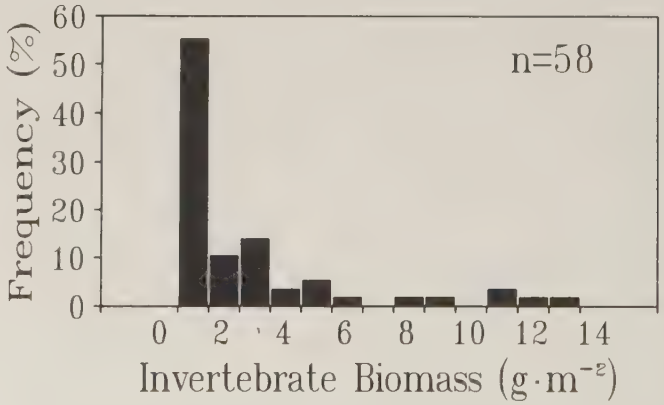

FIG. 4. Frequency histogram of standing phytofaunal biomass (dry mass) found in other published studies. Data represent 58 observations of phytofauna biomass from eight literature sources: Pieczyński (1977), Mittelbach (1981), Biggs and Malthus (1982), Dvořak and Best (1982), Scheffer et al. (1984), Talbot and Ward (1987), and Kornijów (1989, 1990). If published data were given as wet mass, they were transformed to dry mass as 0.15·wet mass.

biomass were greater than the median of literature values, and those found in lac Magog and lac Waterloo were greater than any of these literature values (Table 1). The higher values found in our study probably result from our use of a fine-mesh sieve which retains more of the invertebrate community and the enclosure method of sampling that we used (Downing 1986). Because the sampler tightly encloses a volume, it does not lose loosely attached invertebrates. Downing (1986) found this method to yield population estimates substantially higher (from 2 to 10 times) than other methods, especially for mobile, active organisms such as chironomids, water mites, cladocerans, copepods, and insects.

Phytofauna biomass in these macrophyte beds was frequently dominated by chironomid larvae (Fig. 5) which comprised 30% or more of the total biomass in 8 of 11 sites and made up to 80% of the biomass in lac Waterloo (Fig. 5). Co-dominance of the phytofauna by chironomids and oligochaetes has been reported (Mrachek 1966; Pieczyński 1973; Gerrish and Bristow 1979; Cattaneo 1983; Keast 1984), but oligochaetes made up only 2–11% of the biomass in our samples, being slightly more abundant in eutrophic lakes (p = 0.02). Many authors have reported the dominance of snails in the phytofauna of some lakes (e.g. Biggs and Malthus 1982; Vincent et al. 1982; Kołodziejczyk 1984a; Talbot and Ward 1987). Gastropods represented only 15% of the biomass, on average, in the 11 macrophyte beds (Fig. 5) but made up over 50% of the biomass at two sites (Fig. 5). An overall mean of 18% of the biomass was composed of water mites.

Some organisms such as ostracods, copepods, and cladocerans were present in great numbers but contributed little to the

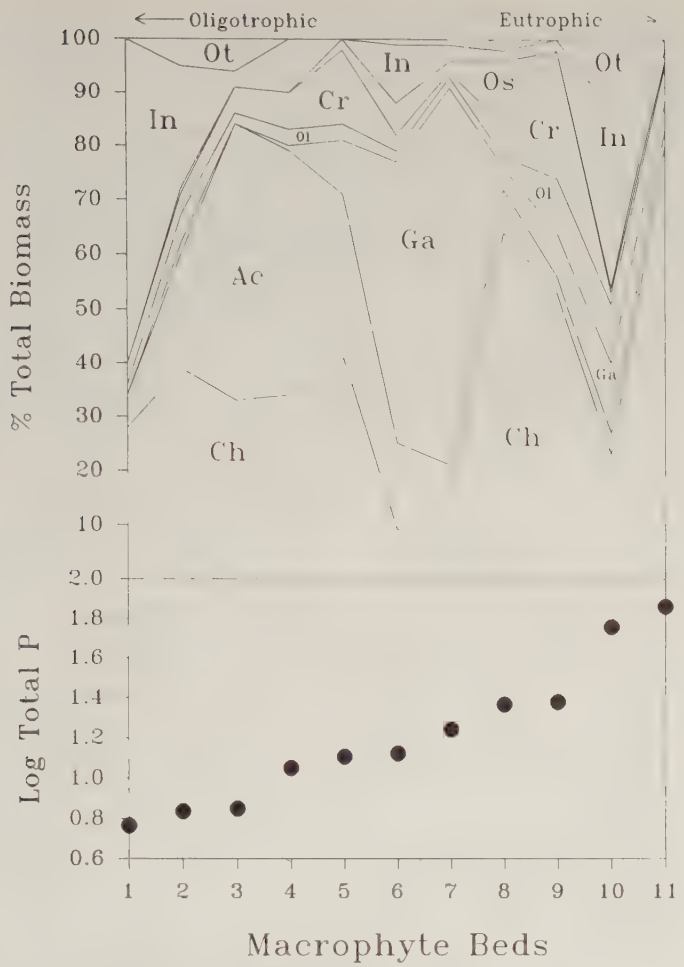

FIG. 5. Phytofauna community composition in the 11 macrophyte beds sampled, as indicated by the percentage contribution of each taxonomic group to the total biomass of invertebrates. Abbreviations of the taxonomic groups are as in Fig. 2 except that Ot = "others". Crustacea includes Copepoda and Cladocera, Insecta includes insects other than Chironomidae, and "others" represents Hirudinea, Amphipoda, and Turbellaria. Macrophyte beds are arranged in increasing order of TP ($\mu g \cdot L^{-1}$; Table 1), and macrophyte bed numbers are as in Fig. 3.

phytofauna biomass due to their small body size. We found more small organisms in our phytofauna samples than other researchers, probably because samples were completely enclosed before removal from the lake (see Downing 1986) and we counted all organisms retained on a fine-mesh sieve (100 μm). Small organisms are seldom considered in studies of littoral communities (e.g. Soszka 1975a; Schramm et al. 1987; Talbot and Ward 1987), probably because the majority of research has been performed with sieves >100 μm. In most cases, however, our data uphold Talbot and Ward's (1987) suggestion that cladocerans and copepods do not contribute significantly to phytofauna biomass. In lac Massawippi, however, these crustaceans were far from negligible, constituting 24% of the phytofaunal biomass. Because of their small body size and high *P/B* ratio (Plante and Downing 1989) and the fact that cladocerans are favored prey of invertebrates and fish (e.g. Goulden 1971; Fairchild 1982; Schramm and Jirka 1989), these organisms are probably very important to the productivity of many littoral zones.

Multiple regression analysis was employed to find how phytofauna biomass covaried with the abundance of food, substrate, and abiotic conditions. In general, the total biomass (TB, micrograms dry mass) of phytofauna found on samples of aquatic macrophytes varied as

TABLE 2. Signs of the significant ($p < 0.05$) regression coefficients associated with biotic variables and macrophyte bed characteristics. BP is the total macrophyte biomass in samples, EPI is the epiphyton biomass, S is the phytoplankton biomass, TP is the total phosphorus concentration, AMB is the areal macrophyte biomass, $\bar{Z}$ is the macrophyte bed mean depth, Z_b is the sampling depth, and date represents the sampling date. BP was transformed to its fourth root and EPI and S to their logarithm. We used a fourth-root transformation for the total biomass of the phytofauna, Copepoda, Cladocera, Gastropoda, Ostracoda, and Acari. A logarithmic transformation was applied to the biomasses of Chironomidae, Trichoptera, and Oligochaeta. A blank space indicates that the particular regression coefficient was not statistically significant ($p > 0.05$).

Taxon	R^2	BP	EPI	S	TP	AMB	$\bar{Z}$	Z_b	Date
Total biomass (Eq. 1)	0.71	+	+		+		−		
Copepoda	0.51	+	+			+	−		
Cladocera	0.67	+	+	+		+			
Chironomidae	0.73	+	+		+	+	−		+
Trichoptera	0.40	+			+			−	
Oligochaeta	0.45		+		+		−		
Gastropoda	0.59	+		+				−	+
Ostracoda	0.52	+		+				−	+
Acari	0.48	+				+	−	−	+

$$(1)\quad (TB)^{0.25} = 11.40\,(BP)^{0.25} + 0.113\,(TP) - 2.01\,(\bar{Z}) + 0.86 \log (EPI) + 3.22$$

($R^2 = 0.70$, $n = 126$, $p < 0.0001$) where BP is grams of macrophyte dry mass, TP is micrograms of total phosphorus per litre of water surrounding the macrophytes, EPI is micrograms of chlorophyll *a* per gram macrophyte dry mass, and $\bar{Z}$ is in metres. Equation 1 suggests that phytofauna increases rapidly with TP, epiphyton concentration appears to have a more dramatic effect at lower EPI, and very small differences in $\bar{Z}$ have a marked influence on the standing invertebrate biomass. Analysis of variance on the residuals of Eq. 1 shows that there was no significant difference ($p > 0.05$) in phytofauna biomass in the two study years.

A regression analysis analogous to Eq. 1 was performed individually for several taxonomic groups: Copepoda, Cladocera, Chironomidae, Trichoptera, Oligochaeta, Gastropoda, Ostracoda, and Acari. The signs of the regression coefficients for these analyses (Table 2) show that the biomass of every group of phytofauna was correlated with the abundance of at least one potential food source (EPI, S, TP) and the quantity of macrophyte substrate from which samples were collected (BP).

It is logical that biomass of fauna dwelling on the surfaces of macrophytes should be correlated with the quantity of substratum sampled (BP). This was the case for nearly every taxon sampled (Table 2), as it has been found in several other ecosystems (e.g. Gerking 1957; Mrachek 1966; Fairchild 1981; Downing 1986; Schramm et al. 1987). AMB in our sampling areas had a positive influence on the biomass of copepods, cladocerans, chironomids, and water mites (Table 2). The presence of dense macrophyte beds in the littoral zone probably influences the phytofauna biomass by increasing the habitat surface and complexity (Mittelbach 1981; Crowder and Cooper 1982; Schramm et al. 1987), offering protection against turbulence (e.g. Bownik 1970) and decreasing predation pressure (Crowder and Cooper 1982; Savino and Stein 1982).

The abundance of potential food sources (EPI, S) was strongly and positively correlated with the biomasses of many taxa (Table 2). This upholds the findings of Cattaneo (1983), Mason and Bryant (1975), and Soszka (1975b) for chironomid and oligochaete biomass. Cladoceran and copepod biomasses were also positively correlated with EPI, possibly because many of these littoral crustaceans can feed on epiphyton (Fryer 1968). Although gastropods are generally considered to be efficient grazers of epiphytic matter (Kołodziejczyk 1984b), we observed no significant correlation between gastropod biomass and EPI. This may be because we measured EPI as chlorophyll *a* which may be a poor estimate of the food available to littoral gastropods (Kołodziejczyk 1984b).

Two measures of phytoplanktonic food, the chlorophyll *a* (S) and TP of the water column, were also strongly correlated with phytofauna biomass (Table 2). Littoral cladocerans not only consume epiphyton but can also be filter-feeders (Fryer 1968; Downing 1981). Prosobranchia (comprising Hydrobiidae, abundant in our samples) can capture particles suspended in the water (Kołodziejczyk 1984b, but cf. Gaevskaya 1966). These results are reiterated by the positive effects of TP indicating that eutrophic lakes yield higher biomasses of chironomids, trichopterans, and oligochaetes (Table 2). Chlorophyll *a* and TP have often been used as predictors of biological activity in other benthic systems (e.g. Hanson and Peters 1984; Rasmussen and Kalff 1987; Rasmussen 1988) and it appears that they can also be used as predictors for phytofauna abundance (Table 2).

$\bar{Z}$ and Z_b were negatively correlated with the biomass of about half the taxa (Table 2). The total phytofaunal biomass and the biomasses of chironomids, copepods, oligochaetes, and water mites were greater in shallower water, regardless of distance from sediments. This suggests that the greater amount of solar radiation and proximity to rich sediments is translated into greater invertebrate abundance in shallower macrophyte beds. The biomasses of trichopterans, gastropods, and water mites were greater closer to the sediment surface than near the water surface. This may occur because the upper parts of macrophytes are younger and may not yet have been as densely colonized as the older leaves closer to the bottom, or invertebrates may avoid the upper parts of plants where wave and turbulence effects are strongest.

The total biomass of the phytofauna was not correlated with sampling date (Table 2). The biomasses of chironomids, gastropods, ostracods, and water mites, however, increased significantly during the 2-mo sampling period. Our results corroborate those of Gerking (1957), Cattaneo (1983), and Kołodziejczyk (1984a) who also found an increase in biomass of gastropods during these months. Total phytofaunal biomass did not vary systematically over time but sampling date had significant effects on community composition.

It is generally believed that some species of macrophytes are more conducive than others to the development of high biomasses of phytophilous invertebrates (e.g. Krecker 1939; Soszka 1975a; Vincent et al. 1982; Rooke 1984, 1986a, 1986b; Talbot and Ward 1987; Cyr and Downing 1988b). When we partitioned macrophyte biomasses among the individual component species in regression analyses, we found that little residual variation in phytofauna biomass could be explained (Table 3). Most R^2 values increased because we were using more independent variables in the analyses, and increases in R^2-values were significant ($p < 0.05$) in 67% of the taxa. Increases in R^2-values were generally very small ($\Delta R^2 \leq 0.05$ in seven of nine cases), however. Only water mites and oligochaetes appeared to show strong preference for certain macrophyte species ($\Delta R^2 = 0.08$). BP alone appeared to account for nearly as much variation in chironomid, gastropod, and total phytofauna biomass as particular macrophyte species (ΔR^2 = NS; Table 3). Most groups of phytofauna showed nearly equal preference for the various macrophyte species in these lakes. Effects of macrophyte community composition might be important, however, where management for specific fish-food groups is the goal.

TABLE 3. Signs of the regression coefficients associated with the biomass of individual macrophyte species in phytofauna samples. Regression analysis was performed as in Table 2, using all the same independent variables, except that BP was partitioned into its component species. ΔR^2 is the difference in R^2 between these multiple regression models and those using only BP. Transformations of the dependent variables are as in Table 2. Macrophyte species: M, *Myriophyllum spicatum*; Va, *Vallisneria americana*; Pa, *Potamogeton amplifolius*; Pp, *Potamogeton praelongus*; Pr, *Potamogeton robbinsii*; Pri, *Potamogeton richardsonii*; Ec, *Elodea canadensis*; Cd, *Ceratophyllum demersum*; P, *Potamogeton* sp.; Hd, *Heteranthera dubia*; N, *Nitella* sp.; Cla, *Cladophora* sp. Significance of changes in R^2-values was determined by an F-test of the ratio of change in model mean-square to the residual mean-square (Gujarati 1978). NS indicates that no significant change in the R^2 resulted from considering the specific composition of macrophyte biomass. An asterisk indicates that R^2 changed significantly ($p < 0.05$).

Taxon	ΔR^2	M	Va	Pa	Pp	Pr	Pri	Ec	Cd	P	Hd	N	Cla
Total	−0.03 NS	+	+	+	+	+	+	+	+				
Copepoda	0.05*			+			+				−		
Cladocera	0.03*	+	+	+		+	+				−		
Chironomidae	−0.02 NS	+	+	+	+	+	+						
Trichoptera	0.02*	+		+		+							
Oligochaeta	0.08*		−								−	+	
Gastropoda	0.01 NS	+	+	+	+			+	+	+			
Ostracoda	0.03*	+	+	+	+			+					+
Acari	0.08*	+	+	+	+	+		+	+				

BP alone appeared to account for nearly as much variation in chironomid, gastropod, and total phytofauna biomass as particular macrophyte species (ΔR^2 = NS; Table 3). Most groups of phytofauna showed nearly equal preference for the various macrophyte species in these lakes. Effects of macrophyte community composition might be important, however, where management for specific fish-food groups is the goal.

This study has shown that numerical abundance and biomass of invertebrates yield dissimilar descriptions of phytofaunal community composition. Studies based on the analysis of numerical abundance of phytofaunal invertebrates yield an inadequate image of community biomass composition and potential energy transfers. We found that variations in community biomass composition of phytofauna could be partially explained by differences in lake trophic status, macrophyte density and composition, and depth of the macrophyte beds we studied. Our analysis of a diverse set of macrophyte beds quantifies the composite effect of macrophyte bed characteristics on phytofauna biomass and underlines, for the first time, the importance of the combined effects of the three primary producers of the littoral zone: macrophytes, epiphyton, and phytoplankton.

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Biotic and Abiotic Determinants of the Diet of Brook Trout,[1] *Salvelinus fontinalis*, in Lakes of the Laurentian Shield

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Lacasse, S., and P. Magnan. 1992. Biotic and abiotic determinants of the diet of brook trout, *Salvelinus fontinalis*, in lakes of the Laurentian Shield. Can. J. Fish. Aquat. Sci. 49: 1001–1009.

From a survey of 12 lakes containing brook trout, *Salvelinus fontinalis*, 12 lakes containing brook trout and creek chub, *Semotilus atromaculatus*, and 13 lakes containing brook trout and white sucker, *Catostomus commersoni*, we built seven multiple linear regression models to account for the mean percent weight of different prey categories in the diet of brook trout. Presence of chub and sucker, zooplankton community structure, sampling date, morphoedaphic index, and the importance of rock outcrops accounted for 88% of the variation in weight of zoobenthos eaten by trout, which was the preferred prey in allopatry. Thirty percent of the variation in weight of zooplankton eaten by trout was explained by the importance of macrophytes and other refuges for fish. Models for amphipods, dipteran pupae, swimming insects, terrestrial insects, and prey-fish explained between 36 and 63% of the variation. The presence of white sucker or an index of their impact (mean length or density of Cladocera) and the characteristics of littoral habitats appeared in six of seven models. Littoral habitats seemed particularly determinant for the inclusion of prey-fish in the diet, more prey-fish being eaten when regufes were abundant. Variables related to lake morphometry and physicochemistry appeared less regularly in the models.

À partir des résultats d'un relevé de 12 lacs contenant de l'omble de fontaine, *Salvelinus fontinalis*, 12 lacs contenant de l'omble de fontaine et du mulet à cornes, *Semotilus atromaculatus*, et 13 lacs contenant de l'omble de fontaine et du meunier noir, *Catostomus commersoni*, nous avons élaboré sept modèles de régression linéaire multiple afin d'expliquer le pourcentage moyen du poids de différentes proies dans l'alimentation de l'omble de fontaine. La présence du mulet à cornes et du meunier noir, la structure de la communauté zooplanctonique, la date d'échantillonnage, l'indice morphoédaphique et l'importance des affleurements rocheux expliquaient 88 % de la variation du poids du zoobenthos consommé par l'omble de fontaine, proie préférée en condition d'allopatrie. La quantité de macrophytes et la présence d'autres refuges pour les poissons expliquaient 30 % de la variation du poids du zooplancton consommé par les ombles. Les modèles élaborés pour les amphipodes, les pupes de diptères, les insectes nageants, les insectes terrestres et les poissons-proies expliquaient de 36 et 63 % de la variation. La présence de meunier noir ou un indice de leur impact (longueur moyenne ou densité des Cladocères) ainsi que les caractéristiques des habitats littoraux sont apparus dans six des sept modèles. Les habitats littoraux semblaient particulièrement déterminants pour l'inclusion des poissons-proies dans le régime alimentaire des ombles, un plus grand nombre étant consommé lorsque des refuges étaient abondants. Les variables reliées à la morphométrie lacustre et aux caractéristiques physico-chimiques sont apparues moins régulièrement dans les modèles.

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Fishes show great flexibility in their foraging behavior in relation to food availability, competition, predation risks, and experience (Dill 1983). Many studies have documented great variability in diet composition between populations of the same species (Martin 1966; Hansson 1985; Magnan 1988; Boisclair and Leggett 1989). However, no study has investigated the factors that might explain such among-population variation. It would be useful to predict the diet composition of fish because food type and food size may influence their growth and yield. For example, Boisclair and Leggett (1989) showed that the percent contribution of some prey category or prey size-class in the diet, and not the mean weight of prey consumed, was correlated with the growth rate of yellow perch, *Perca flavescens*. Also, when salmonids become piscivorous, they experience higher growth rates than when they feed on zoobenthos or zooplankton (Martin 1966; Campbell 1979; Garman and Nielsen 1982). Finally, fish that switch from their original prey to some alternative prey in the presence of competitors or predators undergo slower growth and/or return lower yield (Werner and Hall 1976; Werner et al. 1983; Magnan 1988).

Two kinds of approach can be used to predict the diet composition of a fish species. The mechanistic approach, which yields conceptual models, has been widely employed in the last two decades (Lehman 1986; Schoener 1986; Persson and Diehl 1990). Although conceptual models are absolutely necessary to understand how individual fish select their prey, they are often limited to one prey category, found in one specific microhabitat. The alternative is the empirical approach, based on multiple linear regression models, that has been used mainly to predict the fish yield and the effects of anthropogenic eutrophication (Rigler 1982; Peters 1986). This approach may also be used to identify those factors that influence (directly or indirectly) the diet composition of different populations of a fish

[1]The authors prefer the common name "charr" to designate species of the genus *Salvelinus*.

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species and to elaborate more realistic hypotheses concerning prey selection among different prey categories, in different microhabitats.

There is great among-population variability in diet composition of brook trout, *Salvelinus fontinalis*, in lakes of the Laurentian Shield (Magnan 1988). Generally, brook trout shift their diet from zoobenthos to zooplankton when living sympatrically with introduced species such as creek chub, *Semotilus atromaculatus*, and white sucker, *Catostomus commersoni* (Magnan and FitzGerald 1982; Magnan 1988; Tremblay and Magnan 1991). However, frequent exceptions to this general pattern suggest that factors other than interspecific competition may determine diet. The goal of the present study was to build empirical models to account for this variability in the diet composition of brook trout in 37 oligotrophic lakes of the Laurentian Shield. To this end, we surveyed the feeding habits of brook trout and measured different variables related to the fish community, predator size, prey abundance and size, lake morphometry, physicochemistry, and habitat characteristics of each lake.

Materials and Methods

Study Lakes

The study involved 37 lakes of the Mastigouche Reserve, Québec (46°40′N, 73°20′W). The lakes were selected according to two sampling stratification criteria: fish species composition and lake size. Twelve lakes contained allopatric brook trout populations, 12 contained brook trout and creek chub, and 13 contained brook trout and white sucker, hereafter referred to as "brook trout lakes," "brook trout and creek chub lakes," and "brook trout and white sucker lakes," respectively. Within each fish community type, lakes were distributed as equally as possible among five area-classes: 0–10, >10–20, >20–50, >50–100, and >100 ha. The general characteristics of the lakes are given in Table 1. Northern redbelly dace, *Phoxinus eos*, was present in almost all of the lakes but its presence did not significantly affect the mean yield of trout in the exploited lakes of the Mastigouche Reserve (P. Magnan, unpubl. data). Creek chub and pearl dace, *Semotilus margarita*, were also present in some of the brook trout and white sucker lakes, but gillnet fishing in three of these lakes indicated that the relative biomass of creek chub and pearl dace represented less than 1% of the total fish biomass (P. Magnan, unpubl. data). All lakes were subject to sportfishing, and exploitation was carefully controlled by the Québec government.

Dependent Variables

A mean of 20 stomachs per lake (range 13–25) was sampled from brook trout captured by sport fishermen, during daylight periods, between June 1 and 30, 1989. Sport fishermen had been given a cooler in which they could retain captured brook trout without eviscerating them. Upon the anglers' return, the length of each trout was recorded, and its stomach was removed and preserved in a 10% formaldehyde solution. We assumed that any potential bias related to this sampling technique would be the same in all of the lakes studied. In the laboratory, the wet weights (±1.0 mg) of zoobenthos, amphipods (mostly Gammaridae), zooplankton (mostly Cladocera and Chaoboridae), dipteran pupae, swimming insects (Hemiptera and Coleoptera), terrestrial insects, prey-fish (mostly northern redbelly dace), and other prey found in each stomach were noted.

TABLE 1. General characteristics of the study lakes.

Lake	Surface area (ha)	Mean depth (m)	Conductivity (μS/cm)	Secchi depth (m)
		Brook trout lakes		
Arbout	3.9	3.3	22.3	4.1
Bondi	25.7	8.1	19.1	5.5
Chute Noire	16.9	3.5	21.3	5.2
Diablos	9.8	6.5	18.1	4.5
Ferme	78.6	6.4	26.6	5.9
Gros Ours	35.9	4.8	24.4	6.2
Joncs	14.4	2.8	28.7	5.4
Lafond	43.6	7.9	22.3	6.0
Parker	77.3	8.2	28.7	6.5
Prudent	134.0	9.8	28.7	7.0
Régis	101.9	6.3	25.5	5.3
Vautour	7.2	4.8	26.6	6.6
Mean	45.8	6.0	24.4	5.7
(±1 SD)	(42.5)	(2.2)	(3.7)	(0.9)
		Brook trout and creek chub lakes		
Argent	29.7	10.0	21.3	4.9
Bouteille	254.2	18.2	36.1	6.5
Camp	11.0	6.0	23.4	5.4
Carufel	10.1	6.1	30.8	5.8
Chamberlain	17.1	2.5	23.4	4.3
Cuttaway	39.1	3.7	22.3	5.5
Houde	228.5	17.6	18.1	11.0
Rose	10.9	4.7	31.9	5.0
Smith	7.7	4.7	23.4	4.8
Tête	23.1	7.2	24.4	4.0
Vertnez	40.2	12.1	41.5	7.3
Visons	72.2	8.1	20.2	5.3
Mean	62.0	8.4	26.4	5.8
(±1 SD)	(85.9)	(5.2)	(7.1)	(1.8)
		Brook trout and white sucker lakes		
Bigorne	101.7	3.6	30.8	9.2
Brisé	36.6	3.9	19.1	3.9
Grignon	27.5	7.9	16.0	6.0
Iles	500.8	11.9	28.7	7.8
Joie	10.9	6.5	17.0	4.8
Jones	26.0	8.4	27.6	6.7
Mastigou	158.3	7.4	23.4	4.0
Montour	8.3	2.7	23.4	—
Pimbina	67.2	4.0	22.3	4.4
Plouf	60.9	9.2	24.4	6.8
Sanglier	19.2	4.6	25.5	3.3
Sauterelle	8.1	5.4	21.3	5.0
Vert	17.1	8.7	22.3	8.1
Mean	80.2	6.5	23.2	5.8
(±1 SD)	(133.7)	(2.7)	(4.4)	(1.8)

The mean percent weight of each prey category in the diet of brook trout (Hyslop 1980) was used as the dependent variable in the multiple linear regression analyses.

Independent Variables

All of the independent variables that could vary over a short period (e.g. physicochemistry) were measured within 1–4 d following stomach sampling in a given lake, between 10 and 17 h. Two dummy variables related to creek chub and white

sucker presence were created to describe the fish species composition of the study lakes. Two zero values were given to brook trout lakes, 1 and 0 to brook trout and creek chub lakes, and 0 and 1 to brook trout and white sucker lakes. Fish species distributions were obtained from the ministère du Loisir, de la Chasse et de la Pêche du Québec (MLCP). The mean total length (millimetres) of brook trout was used as a measure of predator size in each lake.

Lake area (square metres), littoral area (0–2 m depth; square metres), lake volume (cubic metres), shoreline length (metres), maximum depth (metres), island area (square metres), and the slope (estimated as the ratio of maximum depth to the square root of lake area; Godbout and Peters 1988) were calculated from bathymetric maps available from MLCP or elaborated during this study.

We estimated the thermal habitat of brook trout because it has been related to the yield of some fish species (Christie and Regier 1988), and therefore it could also influence the food habits of trout. The thermal habitat is defined as the lake benthic area or the water volume with temperature within the fundamental thermal niche of a fish species (Christie and Regier 1988). This thermal niche is estimated as ± 2°C around the final preferendum, skewed 1°C lower to account for the differences between the laboratory estimations and temperatures selected by fish in the field (Christie and Regier 1988). The final preferendum and optimal growth temperature of brook trout averaged 16°C (15–17°C) in the studies of Coutant (1977) and Schlesinger and Regier (1983). We thus established its fundamental thermal niche between 13 and 17°C. A temperature profile was recorded at the center of each lake allowing for the calculation of the thermal habitat volume and area from bathymetric maps.

Water temperature (degrees Celsius), dissolved oxygen (milligrams per litre), pH, and conductivity (microsiemens per centimetre) were measured at 0.5 m from the surface at the deepest point of each lake with a Hydrolab surveyor II (model SVR2), and the water transparency was measured using a 20-cm Secchi disk and an underwater viewer to exclude surface riffle effects. The concentration of total dissolved solids (TDS; milligrams per litre) which is required to calculate the morphoedaphic index (MEI = TDS/mean depth; Ryder 1965) was estimated as 0.67 times the specific conductance at 25°C (microsiemens per centimetre) (Godbout and Peters 1988).

The zooplankton was sampled at three stations in the central part of each lake. At each station, a vertical haul was made in the euphotic zone using a 12-cm Wisconsin plankton net with 80-μm mesh. The euphotic zone depth was estimated as two times the Secchi disk transparency (Holmes 1970; Prepas and Trew 1983). When the euphotic zone was deeper than the entire water column, the hauls were made starting from 0.3 m above the sediments. Organisms were preserved in 95% ethanol solution. In the laboratory, all cladocerans of each sample were identified to genera and measured to the nearest 0.01 m using a dissecting microscope and an ocular micrometer. Other taxa, such as copepods, were not considered in this study because they were almost never found in the stomachs of trout. The length of the cladocerans was measured from the top of the head to the posterior end of the carapace excluding spines. Zooplankton biomass was calculated using the length–weight regressions of Bottrell et al. (1976) for bosminids, *Ceriodaphnia*, *Daphnia*, and *Diaphanosoma*, those of Rosen (1981) for *Polyphemus* and *Sida*, and that of Culver et al. (1985) for *Leptodora*. For *Holopedium*, we used the same regression as for *Daphnia* because suitable regressions for *Holopedium* (Lawrence et al. 1987; Yan and Mackie 1987) require measurements on unpreserved individuals or several measurements per individual. In the present study, the range in length of *Holopedium* overlapped considerably with that of *Daphnia*. Mean length (millimetres), density (number per cubic metre), and biomass (milligrams per cubic metre) of the three principal cladocerans (bosminids, *Daphnia*, and *Holopedium*) and of pooled cladocerans were used as independent variables. All calculations were based on the mean of the three hauls.

We assessed the importance of littoral habitats (0–2 m depth) using visual indices determined by two observers from a boat. The importance of macrophytes and submersed wood was estimated using an index ranging from 0 to 5. The importance of other refuge habitats such as emergent vegetation, bushes, and semisubmersed wood was evaluated by multiplying their percent of littoral occupation with a refuge quality index ranging from 1 to 10. The percent littoral occupation was estimated linearly between two landmarks, on a series of transects covering the entire shoreline, except for lakes larger than 100 ha for which the observations were done on one third of the shoreline. The refuge quality index was judged according to the abundance, tangling, structural complexity, and immersion depth. We also measured the percent littoral occupation of the open habitats (rocks, rock outcrops, beaches, and mud flats).

Statistical Analysis

The stepwise multiple linear regressions were performed using the Statistical Package for the Social Sciences (SPSS X.2). Regressions were made with each of the following functional prey categories as dependent variables: mean percent weight of total zoobenthos (including amphipods), amphipods alone, zooplankton, dipteran pupae, swimming insects, terrestrial insects, and prey-fish. Here, we define a functional prey category as a group of prey having a similar way of life and requiring a specific foraging tactic by the predators. In the stepwise procedure, the independent variable that enters on the first step is the one most highly correlated with the dependent variable; we also forced the entry of other independent variables (highly correlated with the dependent variable) on the first step, followed by a stepwise procedure, a method that sometimes yielded a better model than the usual stepwise procedure. The best models, as indicated by the highest R^2 values and the lowest values of the mean square error associated with the estimate, were retained. Collinearity between the independent variables was evaluated by examination of the pairwise correlation coefficients. The SPSS X.2 regression procedure also uses the "tolerance and minimum tolerance tests" to prevent collinearity problems (Tabachnick and Fidell 1983). When collinearity occurred, each collinear variable was used to build a separate model that was then compared with all of the other models. Residual scatterplots, normal probability plots (Tabachnick and Fidell 1983), and partial residual plots (Larsen and McCleary 1972) were used to determine if the assumptions of the multiple linear regression were satisfied (i.e. normality, linearity, and homoscedasticity of residuals). When these conditions were not fulfilled, different transformations were applied to the data (Montgomery and Peck 1982). The mean percent weight of swimming insects and of prey-fish was log-transformed and the mean percent weight of dipteran pupae and of terrestrial insects

TABLE 2. Diet composition of brook trout in the study lakes. *N*, number of stomachs; ZB, zoobenthos, AM, amphipods; ZP, zooplankton; DP, dipteran pupae; SI, swimming insects; TI, terrestrial insects; PF, prey-fish.

Lake	*N*	Mean percent weight						
		ZB	AM	ZP	DP	SI	TI	PF
Brook trout lakes								
Arbout	20	45.9	0	33.4	5.5	8.6	1.6	0
Bondi	25	53.1	0.1	18.7	6.0	5.3	8.8	0
Chute Noire	20	83.3	2.5	7.5	0.4	0.7	0.5	0
Diablos	23	70.8	1.7	12.2	1.4	0.1	4.3	3.6
Ferme	21	68.7	1.1	26.0	1.4	2.4	0	0
Gros Ours	24	50.0	20.6	14.1	9.2	3.7	0.4	0
Joncs	21	68.1	10.7	8.6	0.3	0.4	0	4.6
Lafond	20	49.3	0.1	27.7	3.2	1.2	10.5	3.6
Parker	22	22.4	3.8	54.6	2.3	12.8	3.1	0
Prudent	21	77.5	1.3	3.4	4.2	4.7	4.1	0
Régis	21	85.2	5.5	1.1	2.1	5.0	0.3	0
Vautour	20	75.6	0	17.6	0.9	2.5	0.8	0
Mean		62.5	4.0	18.7	3.1	4.0	2.9	1.0
(± 1 SD)		(18.6)	(6.1)	(15.0)	(2.7)	(3.8)	(3.5)	(1.8)
Brook trout and creek chub lakes								
Argent	21	42.7	0	4.9	1.9	10.1	33.7	6.6
Bouteille	22	17.7	19.8	55.1	0.2	3.8	0.1	0.4
Camp	15	8.7	0	67.3	1.5	1.6	0.4	9.8
Carufel	16	50.6	0.4	1.3	0.3	7.5	7.2	26.5
Chamberlain	22	63.8	0	0.2	1.5	13.8	13.2	0
Cuttaway	22	50.0	0	24.1	14.9	0.3	6.0	3.5
Houde	20	85.9	0	0	8.5	0.2	0.6	0
Rose	13	28.4	16.5	0	0.2	0	1.8	46.3
Smith	21	38.2	0	53.4	0.9	1.9	0.6	4.3
Tête	22	53.7	0	10.0	12.3	9.7	1.9	12.4
Vertnez	19	3.8	10.0	43.9	7.3	12.4	22.7	0
Visons	19	47.6	0	23.2	18.4	1.8	3.5	5.3
Mean		40.9	3.9	23.6	5.6	5.3	7.6	9.6
(± 1 SD)		(23.5)	(7.3)	(25.0)	(6.5)	(5.1)	(10.6)	(13.8)
Brook trout and white sucker lakes								
Bigorne	21	56.6	0	4.6	36.7	1.5	0	0
Brisé	16	16.2	0	65.4	6.3	0.5	0.9	0
Grignon	21	10.6	0	16.7	30.3	21.5	16.9	3.3
Iles	21	35.5	0	36.6	6.6	13.4	7.2	0
Joie	18	15.6	0	7.6	19.9	32.2	17.5	0
Jones	16	0.5	0	42.4	15.6	6.4	5.5	28.8
Mastigou	21	39.3	0	11.6	5.2	25.3	5.4	0
Montour	18	59.1	0	27.9	4.9	3.1	3.4	0
Pimbina	18	10.1	0	2.1	0.5	10.7	28.8	41.7
Plouf	20	26.4	0	33.4	5.9	24.1	4.3	0
Sanglier	21	22.8	0	18.2	3.5	18.5	18.6	7.5
Sauterelle	20	41.2	0	38.2	1.1	13.1	0.3	3.9
Vert	22	10.3	0	54.1	15.7	8.4	11.6	0
Mean		26.5	0	27.6	11.7	13.7	9.2	6.6
(± 1 SD)		(18.6)	(0.0)	(19.6)	(11.4)	(10.0)	(8.8)	(13.2)

was arcsin-transformed to normalize the residuals and reduce their heteroscedasticity. The usual transformations did not improve the homogeneity of variance of residuals for the mean percent weight of amphipods. This model, obtained with untransformed variables, should thus be considered with caution. One measurement of Secchi disk transparency and five estimates of macrophyte importance were lacking for logistic reasons. These missing values were replaced by the mean values measured on the other lakes. We used the Bonferroni method to correct for multiple tests because the seven models were built with the same set of observations. Each individual regression was thus conducted at a probability of $0.05/n$, where n is the number of regressions, giving a critical value of 0.007 in this study (Cooper 1968).

TABLE 3. Best models predicting mean percent weight of different prey categories in the diet of brook trout. The probability (p) associated with each independent variable, the standard error of the coefficients (SE), the partial R^2 associated with each variable[a], the adjusted R^2, and the standard error of the estimate (S_{xy}) are also listed.

Model	$p > t$	SE	R^2	adj R^2	S_{xy}
Mean % weight of total zoobenthos =			88.0	84.6	9.83
− 5.57	0.6907	13.86			
− 30.10 white sucker	0.0000	4.89	32.7		
− 1.26 sampling date	0.0000	0.23	11.6		
− 22.09 creek chub	0.0000	4.54	0.7		
+ 3.93 morphoedaphic index	0.0000	0.77	9.7		
+ 1.02 rock outcrops	0.0002	0.24	7.5		
+ 87.87 Cladocera mean length	0.0000	16.62	22.6		
− 3.13 Cladocera biomass	0.0002	0.72	8.8		
+ 0.01 *Daphnia* and *Holopedium* density	0.0071	0.00	(5.6)[b]		
Mean % weight of amphipods =			63.4	58.8	3.58
− 46.39	0.0000	9.31			
+ 8.14 pH	0.0000	1.60	36.3		
+ 0.10 rocks	0.0016	0.03	9.2		
+ 0.03 emergent vegetation	0.0189	0.01	9.5		
− 2.85 white sucker	0.0360	1.30	8.4		
Mean % weight of Cladocera and Chaoboridae =			30.0	25.9	17.27
+ 21.42	0.0024	6.53			
− 15.74 macrophyte importance	0.0007	4.24	25.8		
+ 0.18 refuge importance	0.0397	0.09	4.2		
arcsin (mean % weight of *Diptera* pupae) =			36.0	30.1	0.13
+ 0.043	0.6305	0.088			
+ 0.121 white sucker	0.0102	0.044	16.4		
+ 0.030 transparency	0.0346	0.014	10.5		
− 0.001 emergent vegetation	0.0719	0.000	9.1		
arcsin (mean % weight of terrestrial insects) =			54.8	50.7	0.11
− 0.008	0.8962	0.057			
+ 0.001 Cladocera density	0.0000	0.000	37.5		
− 0.024 *Holopedium* biomass	0.0002	0.006	11.1		
+ 0.031 slope	0.0322	0.014	6.2		
log (mean % weight of swimming insects) =			42.7	37.5	0.35
+ 0.972	0.0000	0.144			
+ 0.405 white sucker	0.0019	0.120	20.9		
− 0.023 rock outcrops	0.0076	0.008	12.6		
− 0.099 morphoedaphic index	0.0166	0.039	9.2		
log (mean % weight of prey-fish) =			57.5	50.6	0.38
− 1.767	0.0112	0.654			
+ 0.008 refuge importance	0.0010	0.002	27.5		
+ 0.001 Cladocera density	0.0016	0.000	20.5		
+ 0.251 macrophyte importance	0.0133	0.096	13.9		
− 0.001 bush importance	0.0139	0.000	(2.4)[b]		
+ 0.004 trout length	0.0457	0.002	(2.0)[b]		

[a]Calculated as the standardized regression coefficient times the correlation coefficient between the dependent variable and this independent variable (Tabachnick and Fidell 1983).
[b] Suppressor variable (see text).

Results and Discussion

Feeding Habits of Brook Trout

The results of the present study showed great among-population variability in the diet of brook trout. At the community-specific level, allopatric brook trout fed significantly more on benthic organisms than those living in sympatry with creek chub or white sucker ($F = 9.913$, $p < 0.001$), suggesting that trout shifted to alternative prey in the presence of chub or sucker (Table 2). However, at the lake-specific level, the diet of trout varied as much within each fish community type as among all study lakes (Table 2), suggesting that species composition was not the only factor responsible for the observed variations.

Determinants of the Diet of Brook Trout

High correlations (i.e. $r = 0.7–1.0$) were found between lake area, volume, shoreline length, littoral area, island area, thermal habitat area, and thermal habitat volume, between maximum depth, volume, and thermal habitat volume, between temperature and dissolved oxygen, betwen MEI and maximum depth, between refuge and open habitat importances, between

TABLE 4. Mean length, density, and biomass of total Cladocera and of the three principal cladocerans (bosminids, *Daphnia*, and *Holopedium*) in each fish community. Data are means (±1 SD in parentheses). For each comparison of length, density, and biomass within taxa, means followed by the same letter are not significantly different as determined by an ANOVA followed by a Student–Newman–Keuls multiple-range comparison test ($p > 0.05$). Actual data are presented but analyses were performed on transformed data (log x) for density and biomass. BT, brook trout lakes; CC, brook trout and creek chub lakes; WS, brook trout and white sucker lakes.

	Community	Total Cladocera[a]	Bosminids	*Daphnia*	*Holopedium*
Mean length (mm)	BT	0.94 (0.15) a	0.42 (0.07) a	0.90 (0.10) a	1.09 (0.18) a
	CC	0.87 (0.16) a	0.40 (0.06) a	0.90 (0.15) a	0.97 (0.24) a
	WS	0.70 (0.12) b	0.43 (0.07) a	0.90 (0.08) a	0.80 (0.13) b
Density (N/m^3)	BT	1661 (852) a	132 (81) a	822 (474) a	626 (475) a
	CC	2271 (1398) a	178 (286) a	927 (854) a	1096 (843) a
	WS	2312 (1420) a	843 (920) b	768 (695) a	680 (570) a
Biomass (mg/m^3)	BT	9.43 (5.83) a	0.31 (0.29) a	3.64 (2.81) a	4.50 (4.45) a
	CC	8.77 (5.54) a	0.31 (0.46) a	4.04 (3.19) a	4.12 (4.13) a
	WS	6.60 (4.38) a	1.66 (1.64) b	3.04 (2.59) a	1.75 (1.73) a

[a]Includes bosminids, *Daphnia*, *Holopedium*, and other miscellaneous cladocerans.

density and biomass of bosminids, *Daphnia*, and *Holopedium*, and between biomass of total Cladocera and biomass of *Daphnia* and of *Holopedium*. These independent variables were not considered simultaneously in regression analysis.

The best predictor of mean percent weight of zoobenthos in the diet of trout was the presence of white sucker (−) that accounted for 33% of the variation (Table 3). When brook trout live sympatrically with white sucker, they eat a lower proportion of zoobenthos. This diet shift to alternative prey appears to be caused by a reduction of zoobenthic resources by white sucker (Tremblay and Magnan 1991). White sucker are morphologically and behaviorally better adapted than trout to feed on zoobenthos, while trout are better adapted to feed on alternative prey (Magnan 1989).

It has already been shown that white sucker have a greater impact than creek chub on the food habits of brook trout (Magnan 1988, 1989). This finding was confirmed here on a larger set of lakes by splitting the effects of chub and sucker between two dummy variables. The occurrence of white sucker accounted for one third of the explained variation in mean percent weight of zoobenthos in the diet, while the effect of creek chub, although significant, was negligible (Table 3).

The second most important determinant to enter in the model predicting the use of zoobenthos by trout was the mean length of Cladocera ($R^2 = 0.23$), trout eating more zoobenthos when cladocerans were large (Table 3). The entry of the mean length of Cladocera in the model after the presence of white sucker did not reduce the contribution of the latter, although these variables were intercorrelated ($r = 0.55$). This suggests that there is no problem of collinearity between these two variables. The diet shift of sympatric brook trout to zooplankton had a negative impact on the mean size of Cladocera, trout selecting the larger taxa such as *Holopedium* (Table 4; see also Magnan 1988). Thus, when cladocerans are small, this indicates that trout use or have used cladocerans and therefore that they eat less zoobenthos. In contrast with creek chub, it was also shown that white sucker can feed on zooplankton (up to 30% by weight) later in the summer (Magnan and FitzGerald 1982; Tremblay and Magnan 1991). These results suggest that the mean length of Cladocera could represent an index of the direct and(or) indirect impact of competitors in our model. More specifically, cladoceran length might reflect the "intensity" of the impact of competitors which was not accounted for by the simple use of dummy variables for fish composition in the models.

Sampling date was another determinant of the diet of trout, less zoobenthos being eaten later in June. This could be explained by the progressive seasonal decline of zoobenthic communities that generally occurs in temperate lakes through summer (Mittlebach 1981; Persson 1987; Tremblay and Magnan 1991). Tremblay and Magnan (1991) observed a similar effect of sampling date on the diet of trout, but this impact was spread from May to August. Although we attempted to minimize the influence of sampling date by sampling only in June, the decline of zoobenthic communities during this month might have an impact on the diet of trout.

Lake productivity was also an important predictor of the diet of trout. More zoobenthos was ingested in more productive lakes as suggested by the significant contribution of the MEI in the first model. Johnson (1974) found a positive correlation between zoobenthos standing stock or production and MEI. He also showed the same positive relation with TDS, and many authors report a negative relation between mean depth and zoobenthos abundance or production (Rawson 1955; Johnson 1974; Hanson and Peters 1984; Plante and Downing 1989). It was surprising that the MEI appeared in our model instead of mean depth or TDS, since the latter are generally better predictors than MEI on a limited regional scale (Ryder et al. 1974). The importance of MEI as a determinant in this model was weak and could have been minimized by the fact that white sucker may be more abundant in lakes with high MEI (Trippel and Harvey 1987; Marshall and Ryan 1987). Thus, lakes with high MEI might contain greater zoobenthic resources but also more competitors of brook trout.

Other variables contributing to the first model on zoobenthos were the biomass of Cladocera (−), the importance of rock outcrops (+), and the density of *Daphnia* and *Holopedium* (+) (Table 3). The latter appeared in this model as a suppressor variable (i.e. a regressor that is virtually uncorrelated with the dependent variable but is useful in the model because it suppresses some variance in the other independent variables that is irrelevant for the prediction of the dependent variable; Tabachnick and Fidell 1983).

Sixty-three percent of the variation in the mean percent weight of amphipods eaten by trout was explained by the pH (+), the importance of emergent vegetation (+) and of rocks (+), and the presence of white sucker (−) (Table 3). The pH alone explained up to 36% of this variation. Amphipods are

particularly sensitive to acidity, and their abundance declines as pH decreases (Okland and Okland 1986; Harvey and McArdle 1986). Amphipods are also strongly thigmotactic and react negatively to light. Consequently, they are found in the vegetation or under debris and stones during daytime (Pennak 1953; Edmondson 1959). Such habitats could support higher densities of amphipods, and this might explain why brook trout ate more amphipods when emergent vegetation and rocks were abundant.

The most powerful predictor of mean percent weight of Cladocera and Chaoboridae in the diet of trout was the importance of macrophytes (−), accounting for 26% of the variation (Table 3). Even when the importance of refuges (+) was added to this model, the R^2 was only slightly improved. Neither density, biomass, nor size of Cladocera appeared in this model, suggesting that trout did not respond to the real abundance and size of zooplankton. In some lakes, trout fed on zooplankton even if the size of cladocerans was reduced perhaps because there was less alternative food compared with other lakes. The brook trout population may also be so reduced by competition in some lakes that even if individuals feed largely on zooplankton, the cladocerans would not be significantly affected (Magnan 1988).

Thirty-six percent of the variation in the proportion of dipteran pupae in the diet could be explained by the presence of white sucker (+), the water transparency (+), and the importance of emergent vegetation (−) (Table 3). Dipteran pupae may be easier to find in clearer water for a visual predator like trout and might avoid predation from trout by using the vegetation as a refuge.

The best model predicting the mean percent weight of terrestrial insects in diet of brook trout explained 55% of the variation, particularly owing to the density of Cladocera (+) that accounted for 38% of the variation (Table 3). The biomass of *Holopedium* (−) and the lake slope (+) accounted for the rest of the explained variation (17%). Like mean length of Cladocera in the model predicting the use of zoobenthos by trout, the density of Cladocera might be here an index of the "intensity" of competition: the number of cladocerans was higher in lakes containing chub and sucker, although this tendency was not significant (Table 4).

The inclusion of swimming insects in the diet of trout increased with the presence of white sucker, but it decreased with the abundance of rock outcrops and the MEI, for a total of 43% of explained variation (Table 3).

The multiple regression model using refuge (+) and macrophyte (+) importances, density of Cladocera (+), bush importance (−), and trout length (+) as predictors explained 58% of the variation in the mean percent weight of prey-fish in the diet of trout (Table 3). Trout length and bush importance acted as suppressor variables in this model. Piscivory by trout increased with the importance of refuges (bushes, submersed wood, emergent vegetation, and macrophytes) in the littoral zone. This result may appear paradoxical because northern redbelly dace, the main prey-fish used by brook trout in this study, were significantly more abundant in these habitats during daytime, presumably to reduce the predation risk from tout (Naud and Magnan 1988; M. Proulx and P. Magnan, unpubl. data), and numerous studies have shown an inverse relationship between habitat complexity and predation success (see Gotceitas and Colgan 1989; East and Magnan 1991). Furthermore, prey-fish are often attracted by floating objects (like macrophytes) because the shade reduces their conspicuousness (Helfman 1979). Three nonexclusive hypotheses might explain the positive relationship between habitat complexity and piscivory found in the present study. First, *P. eos* are more abundant in lakes with complex cover. Second, brook trout switch from an active foraging behavior, at low habitat complexity, to a sit-and-wait behavior (sensu Grant and Noakes 1987), at higher habitat complexity. Savino and Stein (1989a) showed that largemouth bass, *Micropterus salmoides*, alter their feeding behavior when vegetative cover increases, switching progressively from an active foraging behavior at low stem density to a sit-and-wait behavior at high stem density. This allowed bass to maintain the same predation success in spite of an increased habitat complexity. In a laboratory study, East and Magnan (1991) noted that brook trout switched from active to sit-and-wait foraging behavior when cover was available for the prey (*P. eos*). Third, brook trout use an active foraging behavior along the cover limit to capture northern redbelly dace that stray into the open areas. Laboratory studies on predation in vegetation have usually used uniform cover instead of a realistic cover where bare zones alternate with vegetation. An exception was the work of Savino and Stein (1989b) who showed that piscivory by bass was higher in pools with 50% cover than in pools with 100% cover, although stem density in covered zones was higher in the former. They observed that bass in 50% cover habitat captured prey-fish that strayed into the open, a behavior also noted for brook trout in the laboratory (East and Magnan 1991).

Conclusions

The empirical models built to explain among-population variations in the diet of trout were especially powerful in predicting the mean percent weight of benthic organisms found in stomachs ($R^2 = 0.88$), which appeared to be the trouts' preferred food resource in allopatry. However, coefficients of determination of the models predicting the mean percent weight of zooplankton, dipteran pupae, swimming and terrestrial insects, and prey-fish in the diet of trout ranged from 0.30 to 0.58. This may result because these prey are used as alternative prey and are then eaten more opportunistically than zoobenthos. Also, because each alternative prey contributed a smaller proportion to the diet of trout than did zoobenthos, its ability to contribute to predictive power was less.

Our models indicated that among the potential factors influencing the diet of brook trout, fish species composition seemed to be the most important. The presence of white sucker was the main determinant of the mean percent weight of zoobenthos, dipteran pupae, and swimming insects in the diet of trout, and also appeared in the model for amphipods. The mean length or the density of Cladocera became important in models predicting the mean percent weight of zoobenthos, terrestrial insects, and prey-fish which were eaten. Since cladoceran size and abundance were affected by the presence of chub and sucker (through their direct utilization of zooplankton and(or) their indirect impact on trout diet), these variables could represent the "intensity" of the impact of trout competitors. Although competition between brook trout and nonsalmonid species explained the largest part of the among-population variability in the diet composition of trout, environmental parameters were also significant. More specifically, characteristics of the littoral habitats appeared in six of seven regression models and seemed particularly important for determining the inclusion of prey-fish

in the diet of trout. The results of this study thus provide evidence that not only biotic but also abiotic variables must be considered to understand prey selection by fishes. The empirical approach used here must be viewed as complementary to the mechanistic approach in providing support for the inclusion of habitat parameters (e.g. refuges for fish) in more conceptual models.

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On the Chemical Form of Mercury in Edible Fish and Marine Invertebrate Tissue

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Bloom, N. S. 1992. On the chemical form of mercury in edible fish and marine invertebrate tissue. Can. J. Fish. Aquat. Sci. 49: 1010–1017.

Total mercury, monomethylmercury (CH_3Hg), and dimethylmercury ($(CH_3)_2Hg$) in edible muscle were examined in 229 samples, representing seven freshwater and eight saltwater fish species and several species of marine invertebrates using ultraclean techniques. Total mercury was determined by hot HNO_3/H_2SO_4/BrCl digestion, $SnCl_2$ reduction, purging onto gold, and analysis by cold vapor atomic fluorescence spectrometry (CVAFS). Methylmercury was determined by KOH/methanol digestion using aqueous phase ethylation, cryogenic gas chromatography, and CVAFS detection. Total mercury and CH_3Hg concentrations varied from 0.011 to 2.78 $\mu g \cdot g^{-1}$ (wet weight basis, as Hg) for all samples, while no sample contained detectable $(CH_3)_2Hg$ (<0.001 $\mu g \cdot g^{-1}$ as Hg). The observed proportion of total mercury (as CH_3Hg) ranged from 69 to 132%, with a relative standard deviation for quintuplicate analysis of about 10%; nearly all of this variability can be explained by the analytical variability of total mercury and CH_3Hg. Poorly homogenized samples showed greater variability, primarily because total mercury and CH_3Hg were measured on separate aliquots, which vary in mercury concentration, not speciation. I conclude that for all species studied, virtually all (>95%) of the mercury present is as CH_3Hg and that past reports of substantially lower CH_3Hg fractions may have been biased by analytical and homogeneity variability.

Le mercure total, le mercure-monométhyl (CH_3Hg) et le mercure-diméthyl ($(CH_3)_2Hg$) ont été dosés dans 229 échantillons de muscles comestibles provenant de sept espèces de poissons d'eau douce, de huit espèces de poisson de mer et de diverses espèces d'invertébrés marins à l'aide de techniques limitant la contamination au minimum. La teneur en mercure total a été déterminée par digestion au HNO_3/H_2SO_4/BrCl, réduction par le $SnCl_2$, passage sur l'or et dosage par spectrométrie de fluorescence atomique de vapeurs froides (SFAVF). La teneur en mercure-méthyl a été déterminée par digestion KOH/méthanol, éthylation en phase aqueuse, chromatographie cryogène en phaque gazeuse et SFAVF. Les concentrations de mercure total et de CH_3Hg de tous les échantillons se situaient entre 0,011 et 2,78 $\mu g \cdot g^{-1}$ (Hg, poids humide) et aucun échantillon ne contenait de quantité décelable de $(CH_3)_2Hg$ (<0,001 $\mu g\ Hg \cdot g^{-1}$). La proportion de mercure total (sous forme de CH_3Hg) se situait entre 69 et 132 %, l'écart type relatif des dosages, effectués à cinq reprises s'élevait à 10 % environ. Pratiquement toute cette variabilité peut être expliquée par la variabilité analytique des dosages du mercure total et du CH_3Hg. Les échantillons mal homogénéisés sont ceux qui présentaient la plus grande variabilité, notamment par ce que le mercure total et le CH_3Hg avaient été dosés à partir des fractions distinctes dont la concentration, et non la spéciation, était variable. L'auteur conclut que, pour toutes les espèces étudiées, pratiquement tout le présent (>95%) l'était sous forme de CH_3Hg et que les rapports antérieurs faisant état d'une proportion passablement inférieure de CH_3Hg était sans doute biaisés.

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Interest in the biogeochemical cycle of mercury in the environment has dramatically increased in recent years because of observations that fish tissue mercury levels are elevated in acid-impacted pristine lakes (Bloom et al. 1991; Grieb et al. 1990; Wiener and Stokes 1990; Bjorklund et al. 1984). Researchers have long known that most of the mercury in fish tissue is in the methylated form (Westöö 1967), but debate continues to this day as to whether the value is closer to 80% (WHO 1990), 90% (Bishop and Neary 1974), or 100% (Grieb et al. 1990) and whether the mercury is in the monomethyl or dimethyl form.

Most determinations of monomethylmercury (CH_3Hg) in tissues are performed by variations of the acid halide extraction plus gas chromatographic (GC) separation method developed by Westöö (1967). These methods are typically tedious and complex, as they seek to extract and purify the methylmercury bound to tissue into a small volume of solvent suitable for GC injection (Horvat et al. 1990; Cappon and Smith 1977; Zelenko and Kosta 1973). Unfortunately, multiple steps are required, each of which can result in losses in yield. In addition, the electron capture (EC) detector which actually measures the halide rather than the mercury atom is prone to interference from the many known and unknown halogen-containing species in the typical laboratory.

Other methods currently being employed for the determination of CH_3Hg in tissues include aqueous phase ethylation with cryogenic GC separation and atomic fluorescence detection (Bloom 1989), headspace analysis of volatile CH_3Hg halides (Lansens and Baeyens 1990), liquid chromatography (Holak 1982), and selective reduction (Magos 1971). These methods, too, have potential problems because they either lack true species specificity or because interference diminishes yields. Finally, all methods which employ an acid extraction step convert dimethylmercury ($(CH_3)_2Hg$) to the monomethyl

form, thus diminishing the speciation information gained from that analysis.

Total mercury methods are generally much more straightforward and well tested, giving an element-specific measure of mercury content by atomic spectroscopic or neutron activation techniques (Bloom and Crecelius 1987; WHO 1976; Magos 1971). Total mercury determinations, however, are at greater risk of contamination of samples during handling because of the ubiquity of inorganic mercury in the laboratory (Gill and Fitzgerald 1985).

The potential for contamination of total mercury analysis, combined with low yields for methylmercury analysis, can lead to systematic underestimation of percent methylmercury in samples. Also, when both methylmercury and total mercury are measured on different subaliquots of the same sample, the combined analytical variability can easily increase to the point where no meaningful judgement of percent methylmercury can be made. This problem is greatly exacerbated by poor sample homogenization prior to aliquoting. Biases such as these may go unnoticed, more often at routine and government testing laboratories than at research institutions, because of the large sample volumes and lower QA requirements for monitoring work.

A potentially significant bias sometimes seen in monitoring data is the rejection of "high" percent methylmercury values more often than equally low values. This is based upon the intuitive but statistically mistaken notion that values of greater than 100% methylmercury are impossible. This selection process will, of course, unjustifiably skew the mean fraction of methylmercury to lower values. Careful inspection of such data often indicates that all of the values are randomly distributed around 100%, primarily because of analytical error. Thus, data may sometimes be summarized as reporting that methylmercury values were, for example, 85–100% of the total, while in fact the results indicated 85–115% (N. S. Bloom, pers. obs.). This is a significant statistical misinterpretation.

The purpose of this paper is to summarize the results of our past 2 yr of fish speciation analysis. Emphasis will be placed not upon particular concentrations, but upon the fraction of mercury in the methylated form, the statistical distribution of this parameter, and how this distribution may be influenced by analytical variables. The influence on the mean of rejecting analytically high percent methylmercury results will also be discussed.

All of these results have been generated by the recently developed technique of aqueous phase ethylation, followed by cryogenic GC separation, and cold vapor atomic fluorescence spectrometry (CVAFS) detection (Bloom 1989). The advantages of this method are that (1) there are no detectable blanks, (2) it is truly species specific, and (3) with sufficient analytical care, the yield is always >95%. These features allow more unambiguous interpretation than with other methods.

Materials and Methods

Sample Collection

Tissue samples were collected and analyzed, over a period of 2 yr, for several agencies, on unrelated projects. Marine species were collected in Puget Sound bays (English sole (*Pleuronectes vetulus*, formerly *Parophrys vetulus*), Dover sole (*Microstomus pacificus*), striped seaperch (*Embiotoca lateralis*), red rock crab (*Cancer productus*), Dungeness crab (*Cancer magister*)) or purchased from the fresh fish market (spot shrimp (*Pandalus platyceros*), weathervane scallops (*Patinopecten caurinus*), blue marlin (*Makaira nigricans*), lingcod (*Ophiodon elongatus*), swordfish (*Xiphias gladius*), sablefish (*Anoplopoma fimbria*), and chinook salmon (*Oncorhynchus tshawytscha*)). Market fish, with the exception of swordfish and blue marlin, were from Alaskan waters. Freshwater species (largemouth bass (*Micropterus salmoides*), yellow perch (*Perca flavescens*), northern pike (*Esox lucius*), and white sucker (*Catostomus commersoni*)) were collected from several remote midwestern lakes and from one mercury-contaminated site. All fish were randomly distributed in size and age.

For the purpose of this paper, the tissues have been separated into the following two categories: (1) those which were dissected and homogenized by the collecting agency and (2) those which were sent to Brooks Rand, Ltd., whole, and were subsequently dissected and homogenized using trace metal clean techniques prior to analysis. Also in the second category are samples which were purchased fresh at the fish market.

No attempt was made to control or record the laboratory conditions under which the samples processed by the collecting agencies were held. In most cases, we received roughly homogenized (~2- to 4-mm pieces) fish muscle tissue, frozen in glass jars with teflon-lined lids. In one case, noted later, the fish were homogenized whole to an equally coarse degree. No attempt was made to homogenize these samples further.

All tissues processed at Brooks Rand, Ltd. were thoroughly rinsed with low-mercury (<1 ng·L^{-1}) water, and an outer section approximately 0.5 cm thick was cut off and discarded prior to taking the sample. A section of clean muscle tissue of approximately 30 g was then removed and homogenized by chopping (to about 1-mm pieces) using clean stainless steel tools and a teflon cutting board. All operations were performed while wearing cleanroom gloves, in an atmosphere containing <10 ng total Hg·m^{-3}. Once the sample was homogenized, it was placed into an acid-cleaned teflon vial and frozen until digestion.

To directly assess the potential for contamination by collecting agency handling of the samples, duplicate sections of three fish, cleanly dissected and homogenized at Brooks Rand, Ltd., were also sent to another contract research laboratory for similar processing. Approximately 100-g fillets of muscle tissue were sent frozen in polyethylene bags. Upon arrival at the second laboratory, they were thawed and an approximately 5-g aliquot dissected out. This aliquot was then homogenized in a stainless steel blender. The homogenates were placed into acid-cleaned glass vials with teflon lids and returned to Brooks Rand, Ltd. frozen. No special attempt was made at the second laboratory to handle the samples in a rigorously trace-metal-free manner.

Analytical Methods

For total mercury analysis, sample aliquots of approximately 2 g were accurately weighed into 100-mL volumetric flasks, 7 mL of HNO_3 and 3 mL of H_2SO_4 added (Bloom and Crecelius 1987), and the opening capped with acid-cleaned glass marbles (the marbles serve as pressure relief valves during the reflux step to follow). The samples were allowed to digest cold overnight and then were slowly brought to a refluxing boil on a hot plate the following day. They were gently refluxed for 3 h, or until the solution was clear, and the production of brown fumes

TABLE 1. Laboratory performance on standard reference materials over a 2-yr period (1989–91) as part of standard QA procedures on tissue analysis work (all results in $\mu g \cdot g^{-1}$ as Hg, dry weight basis).

Standard material	Total mercury			Methylmercury			% as methylmercury	
	Mean	SD or CI[b]	*N*	Mean	SD or CI[b]	*N*	Mean	SD[b]
NBS-5O (tuna[a])								
This laboratory	0.906	0.073	23	0.856	0.073	25	96	14
Recommended	0.95	0.11		0.84	0.17	—	(85–95)	
DORM-1 (dogfish)[c]								
This laboratory	0.795	0.041	23	0.745	0.052	26	94	7
Certified	0.798	0.074	—	0.731	0.060	—	(77–109)	
MA(S)-86/TM (shrimp)[d]								
This laboratory	1.84	0.06	2	0.059	0.007	2	3.2	—
Certified	1.79	0.24	—	--	—	—	—	—
Horvat et al. 1990	1.87	0.07	3	0.084	0.005	3	4.5	—
IAEA-350 (tuna)[d]								
This laboratory	4.62	0.23	4	4.01	0.17	2	86.8	—
Certified	4.99	0.26	—	—	—	—	—	—
Horvat et al. 1990	5.06	0.06	4	3.34	0.15	3	66.0	—
MA-M-2/TM (mussel)[d]								
This laboratory	0.95	0.06	4	0.057	0.004	2	6.0	—
Certified	0.95	0.10	—	—	—	—	—	—
Horvat et al. 1990	0.83	0.02	6	0.058	0.004	3	7.0	—
MA-A-1/TM (copepod)[d]								
This laboratory	0.28	0.02	4	0.017	0.001	2	6.1	—
Certified	0.28	0.01	—	—	—	—	—	—
Hovat et al. 1990	0.274	0.007	3	0.018	0.002	3	6.6	—

[a]Produced by National Bureau of Standards (NBS). Value for mercury in the material is listed as recommended, rather than certified, because requisite multiple independent methods of analysis were not available at the time of issue.

[b]Standard deviation for laboratory analysis; 95% confidence band for certified values.

[c]Produced by the National Research Council of Canada (NRCC).

[d]International Atomic Energy Agency (IAEA) standards were analyzed blind as part of an interlaboratory intercalibration.

had diminished. After cooling, they were diluted to exactly 100 mL with low-mercury water and 5 mL of 0.2 N BrCl (Bloom and Crecelius 1983). Appropriate-sized aliquots (25–1000 μL) of the digestate were analyzed by $SnCl_2$ reduction, dual gold amalgamation, and (CVAFS (Bloom and Crecelius 1983; Bloom 1989). The detection limit, under the conditions described, was about 0.001 $\mu g \cdot g^{-1}$ (wet weight basis).

For methylmercury analysis, sample aliquots of approximately 0.5 g were accurately weighed into 22-mL teflon vials and 10 mL of 25% KOH in methanol added (Bloom 1989). The caps were replaced and the vials vigorously shaken and then placed in an oven at 70°C for 3 h to complete the digestion. After cooling, the digestates were diluted with methanol to a mark on the vials equal to 18.2 mL. Small aliquots of the digestate (10 μL) were analyzed by aqueous phase ethylation, cryogenic GC separation, and CVAFS detection (Bloom 1989). The detection limit under these conditions is about 0.0005 $\mu g \cdot g^{-1}$ (as Hg) for CH_3Hg and 0.0001 $\mu g \cdot g^{-1}$ (as Hg) for $(CH_3)_2Hg$.

Similar QA/QC procedures were followed on all projects where fish tissue data were collected. These include the analysis of blanks and laboratory triplicates once every 10 samples and the analysis of National Bureau of Standards (NBS) or National Research Council of Canada (NRCC) certified standard tissues at least once for every 20 samples. All calibration for methylmercury analysis was performed by the method of standard additions to compensate for potential inhibition of the ethylation due to the tissue matrix. The results of standard additions were always compared with standards run in deionized water and found to be insignificantly different.

In all cases where methylmercury and total mercury differed by more than 35%, both samples were redigested in duplicate and the obviously errant value discarded. The rest of the data were then pooled to form a mean for the total and methylmercury values. This procedure was applied in 11 of 237 cases, for a frequency of 4.6%. In 9 of the 11 cases, an obviously incorrect value was found: generally either a too high Hg_t (contamination) or a too low methylmercury value (pipetting error). In the remaining cases, the triplicate analyses were consistent, and the mean results were used.

In addition to the analysis of certified standard tissues, four laboratory intercomparison tissues from International Atomic Energy Administration (IAEA) sent to our laboratory for blind analysis using these techniques. Results are compared with the exhaustive analysis of Horvat (1991) who employed several distinct and independent analytical techniques. These included instrumental neutron activation analysis (INAA) and cold vapour atomic absorption spectrometry (CVAAS) for total mercury and extraction, volatilization, or distillation followed by EC GC for methylmercury.

Results and Discussion

Quality Assurance

Presented in Table 1 is a summary of all of the standard aquatic tissues analyzed for mercury speciation over the time

TABLE 2. Percent methylmercury in freshwater and saltwater fish muscle tissue. Three marine invertebrates are included for comparison (A = processed and analyzed under ultraclean conditions; B = one half sample processed under ultraclean conditions and the other half at another, uncontrolled contract laboratory (see Table 4); C = processed by the client and analyzed as received (frozen, glass bottles)).

Species	Processing	Total mercury ($\mu g \cdot g^{-1}$) Mean	Percent as methylmercury Mean	SD	N[a]	% fat[b]
Lingcod (*Ophiodon elongatus*)	A	0.157 0.004	94	6	5	1.1
Chinook salmon (*Oncorhynchus tshawytscha*)	A	0.039 0.002	105	6	10	8.9
Blue marlin (*Makaira nigricans*)	A	0.716 0.023	95	9	10	—
Largemouth bass (*Micropterus salmoides*)	A	(0.087–0.364)	99	9	30	3.7
Weathervane scallop[c] (*Patinopecten caurinus*)	A	0.037 0.001	97	6	5	0.7
Spot shrimp (*Pandalus platyceros*)	A[c]	0.020 0.002	100	16	5	1.8
Dungeness crab (*Cancer magister*)	A	0.109 0.010	102	6	3	0.7
Dover sole (*Microstomus pacificus*)	B	0.111 0.019	98	14	10	0.5
Sablefish (*Anoplopoma fimbria*)	B	0.297 0.037	112	14	9	13.0
Swordfish (*Xiphias gladius*)	B	0.428 0.026	100	10	10	3.4
English sole (*Pleuronectes vetulus*)	C	(0.053–0.163)	95	13	24	0.5
Yellow perch (*Perca flavescens*)	C	(0.160–0.260)	99	16	19	—
Northern pike (*Esox lucius*)	C	(0.300–1.444)	103	10	3	0.7
White sucker (*Catostomus commersoni*)	C	(0.090–0.340)	96	10	3	2.4
Striped seaperch (*Embiotoca lateralis*)	C[d]	(0.036–0.372)	86	16	27	0.8
Red rock crab	C	(0.014–0.251)	91	19	25	0.7

[a]N = analytical replicates of a single sample (individual digestions) for values expressed as a mean and standard deviation; N = number of individuals for values expressed as a range.
[b]Pennington (1989).
[c]Three individuals were pooled and homogenized to make the sample.
[d]Whole-fish values.

covered by this paper (1989–91). Approximately 25 analyses each have been made of NBS-50 (NBS, dessicated tuna) and DORM-1 (NRCC, dogfish muscle). These results show extremely close correspondence to the certified values both for the mean and the range, indicating that the results generated by the techniques of this paper are intercomparable both over time and between analysts. The analytical variability for the NBS-50 tuna (RSD percent methylmercury = 14.6%) is about twice that for the DORM-1 (relative standard deviation (RSD) percent methylmercury = 7.5%). This is probably because the NBS-50 samples came from six different cans, supplied by sponsors over a 3-yr period, while all of the DORM-1 aliquots were taken from a single bottle.

The values in Table 1 serve to illustrate the "best-case" precision and accuracy of the method under actual long-term routine use. In particular, the 7.5% RSD in percent methylmercury, in the case of a truly homogeneous sample, will be used as a guide when assessing the variability in other data sets throughout this paper. Greater variability in other data sets will be taken as an indication either of real between-sample differences or variability due to sample heterogeneity.

Also contained in Table 1 are results of the "blind" intercalibration exercise conducted with Dr. Milena Horvat of the "J. Stefan" Institute (Ljubjana, Slovenia). The results obtained by Brooks Rand, Ltd. are generally within 5–10% of those of Dr. Horvat, as well as the IAEA certified values (Horvat et al. 1990). Because several very different methods were employed to obtain Dr. Horvat's results, they serve as a strong confirmation that the aqueous phase ethylation technique does correctly identify the speciation of mercury in tissues.

Total and Organomercury Compounds in Individual Fish Species

Total mercury and the percentage of the total present as CH_3Hg for several marine and lacustrine species are presented

TABLE 3. Statistical breakdown of percent methylmercury data as a function of processing technique.

Sample handling	*N*	Mean	SD	Skewness	95% CI
Whole data set					
Actual reported data	239	96.3	14.5	0.165	94.4–98.2
High values reduced to 100%	239	92.2	9.5	−1.22	91.0–93.4
Processed by collecting agency					
Actual reported data	139	94.9	16.5	0.260	92.1–97.7
High values reduced to 100%	139	90.4	10.6	−0.93	88.6–92.2
Ultraclean processing					
Actual reported data	90	98.5	10.5	0.466	96.3–100.7
High values reduced to 100%	90	95.2	6.3	−1.14	93.9–96.5

in Table 2. $(CH_3)_2Hg$ was never detected in any of the samples tested (<0.001 $\mu g \cdot g^{-1}$ as Hg). Although these samples were subjected to varying degrees of handling rigor, it is clear that in every case, nearly all of the mercury present was in the form of methylmercury, with a variability around the mean that can be largely associated with the analytical error. Contrary to the assertion of Bishop and Neary (1974) that blue marlin is anomolously low (i.e. 25%) in methylmercury, we observed 95 ± 9% (N = 10) for a single fish. Interestingly, even the small sampling of nonfish species, including shrimp, crabs, and scallops, also shows close to 100% mercury in the methylated form. Excluding data for striped seaperch, which were whole fish homogenates, the mean of the mean methylmercury values in the table is 99.1 ± 5.1% (N = 15 species).

The striped seaperch are included only as a qualitative illustration of differences which might arise when whole fish samples are compared with edible muscle tissue. The mean fraction methylmercury is lower (86%) and the variability is higher than is the case for muscle tissue. This discrepancy probably represents the combined effects of greater sample heterogeneity (the material was coarsely chopped, containing scales, bone fragments, etc.) and the fact that some organs, such as livers and kidneys, contain higher levels of inorganic mercury than does muscle tissue (Boudou and Ribeyre 1983; Lockhart et al. 1972) because of internal demethylation and elimination mechanisms.

Since a wide representation of freshwater and saltwater species, free swimmers, and bottom feeders show the same results, it is likely that all fish contain virtually all of their muscle tissue burden of mercury as CH_3Hg. This is supported by data reported elsewhere (Grieb et al. 1990; Lockhart et al. 1972), although the assumption that only about 80% is in the methylated form still persists in regulatory circles (WHO 1976).

Although most of the nonfish species analyzed to date by this laboratory also show all mercury in the monomethyl form, this is not the case for aquatic animals in general. For example, Horvat et al. (1990) reported only about 10% of the total mercury in mussels, copepods, and shrimp from the IAEA standards program (Table 1) as methylmercury, which was corroborated by our own analysis. Most of the remainder in these tissues is, surprisingly, reported by Horvat et al. (1990) to be ethylmercury. We have also found that clams contain only about half their mercury burden in the methylated form (50.1 ± 24.6%, N = 19). These data are not presented, however, because the collecting agency homogenized various species of clam together, making interpretation difficult.

Relationship to Fat Content

Although this study was not conducted in a manner which allows a statistical correlation of fish fat content with methylmercury levels (no fat measurements were made), data for typical species fat content (Pennington 1989) are provided for qualitative comparison. Although the percent fat in these fish varies by about a factor of 25, there is no discernable trend of greater methylmercury levels in fish of higher fat content. This is not surprising considering the low octanol–water distribution coefficient (K_{ow}) for mercury (Bienvenue et al. 1984) compared with traditionally fat-soluble compounds such as DDT and PCB (Veith et al. 1980). Using the typical log–log relationship of K_{ow} and bioconcentration factor for lipid-soluble species (Veith et al. 1980), it is not possible to explain the 10^6–10^7 bioconcentration factor of methylmercury (Bloom et al. 1991). Thus, some other mechanism such as protein affinity must be invoked.

Statistical Distribution of Percent Methylmercury in Fish Tissue

All the speciation data for mercury in fish tissues collected by this laboratory (including shrimp, scallops, and crabs, whose mean methylmercury values are indistinguishable from 100%) were divided into the following two categories: those dissected and homogenized using ultraclean techniques by this laboratory and those supplied to this laboratory already dissected and homogenized by a variety of collecting agencies. In both cases, the mean percent mercury in the methylated form is close to 100% (Table 3).

The samples processed outside the laboratory are slightly lower, 95% compared with 99%, a factor which probably is attributable in part to a low level of inorganic mercury contamination. The fact that the whole perch samples are included in this data set accounts for about a 1% drop in the mean percent methylmercury. Also in Table 3, the statistics have been recalculated with the assumption that all percent methylmercury values greater than 100% are reduced to exactly 100%. With these data, such a misrepresentation results in an approximately 5% decrease in the reported mean percent methylmercury. The magnitude of the error appears to increase with the variability of the data set.

More dramatic than the observed difference in the means is the much greater variability in the data set for samples processed by the collecting agencies compared with those processed under ultraclean conditions (Fig. 1). A comparison of

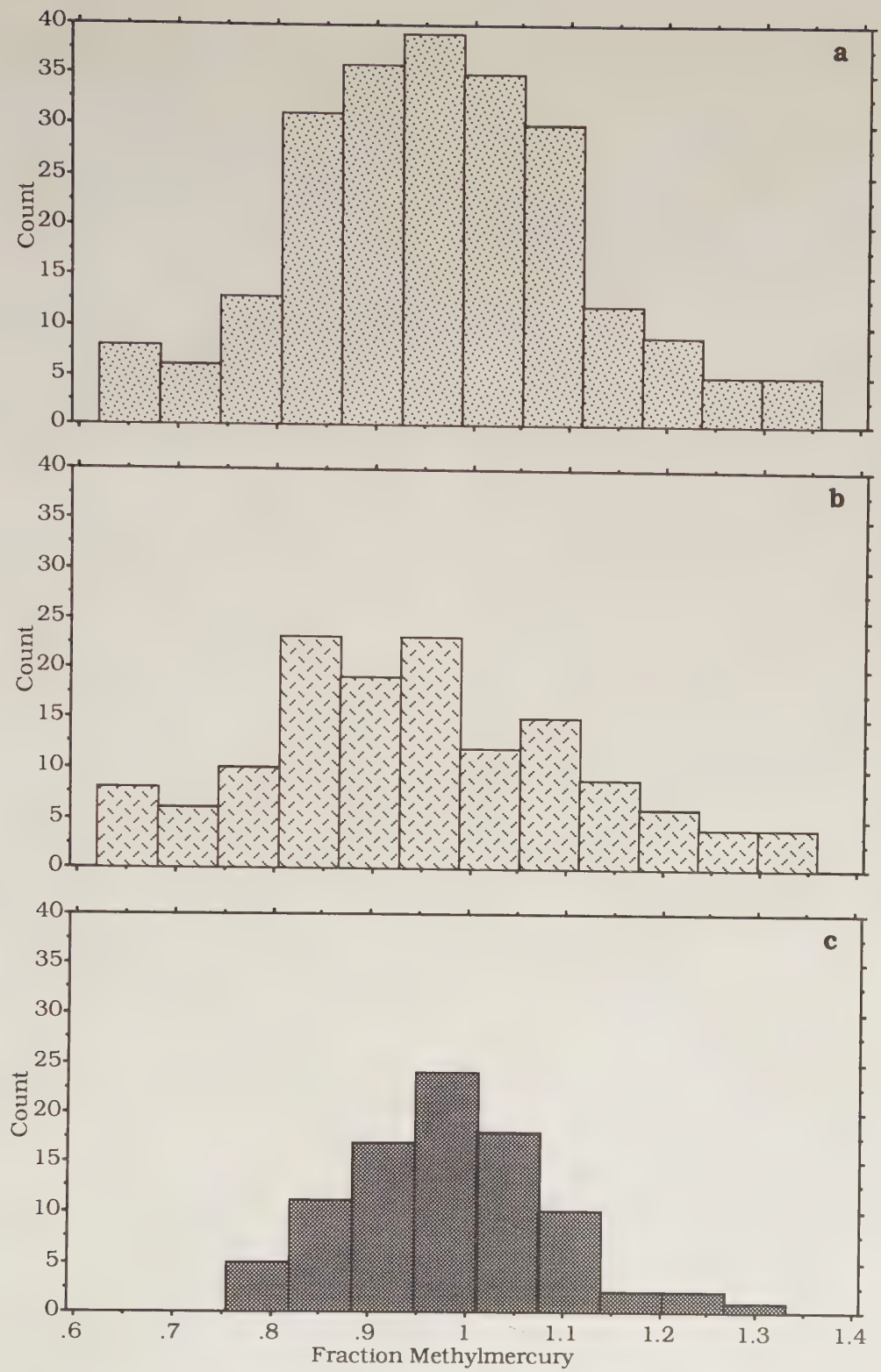

FIG. 1. Frequency diagrams for occurrence of fraction methylmercury in aquatic tissue samples. (a) All samples (N = 229); (b) samples processed by collecting agency laboratories (N = 139); (c) samples processed under ultraclean conditions (N = 90).

the results from the coarsely homogenized samples supplied by the collecting agencies with the more finely processed ones from our laboratory, and the completely pulverized SRMS, leads to the conclusion that much of this variability is not due to actual between-sample differences in chemical form. Instead, there is an artificial variability superimposed by the determination of total mercury and methylmercury from separate aliquots, which may vary in concentration, but not in fraction methylmercury. This variability becomes particularly pronounced in coarsely homogenized samples which contain tissues of differing mercury content (i.e. whole fish, or those with large fatty areas).

Several fish samples were split at our laboratory, and one half was dissected and homogenized under clean conditions, while the other half was sent to another laboratory for homog-

TABLE 4. Comparison of ultra-clean dissection, homogenization, and storage, with the normal protocols for biological (non-tracemetal) sample handling at another research laboratory.

Species	Total mercury ($\mu g \cdot g^{-1}$)			Methylmercury ($\mu g \cdot g^{-1}$)		
	Mean	SD	*N*	Mean	SD	*N*
Dover sole						
Ultraclean handling	0.127	0.011	5	0.115	0.016	5
Less rigorous handling	0.095	0.004	5	0.101	0.011	5
Sablefish						
Ultraclean handling	0.268	0.025	5	0.301	0.032	5
Less rigorous handling	0.326	0.019	5	0.356	0.034	4
Swordfish						
Ultraclean handling	0.441	0.020	5	0.450	0.031	5
Less rigorous handling	0.415	0.025	5	0.402	0.046	5

enization using "ordinary" (not rigorously clean) technique. These results (Table 4) show no significant contamination or alteration of the fraction methylmercury as a function of less rigorous sample handling techniques. This does not eliminate the possibility that some laboratories may contaminate samples with total mercury, thus decreasing the observed fraction of methylmercury, but it does suggest that it is not a general problem.

Small differences were observed in the total mercury content of these sample splits from the same fish. This probably occurred because the sample homogenized at Brooks Rand Ltd. was an entire half fillet (about 100 g), while the second laboratory extracted only approximately a 5-g aliquot from a narrow region of their half fillet. However, the processing of a small aliquot, which is more easily contaminated than a large one, still did not result in perceptible changes in speciation. These results are reinforced by the absence of any decrease in the observed percent methylmercury in samples of low total mercury concentration (<0.05 $\mu g \cdot g^{-1}$) compared with those of higher concentrations (0.5–3 $\mu g \cdot g^{-1}$).

Conclusions

The mercury content for the muscle tissue of all fish appears to be virtually all (99%) in the form of CH_3Hg. Whether the actual level is 98 or 100% is impossible to assess further at this time, given the analytical uncertainty in the methods available. It also appears that many other, although not all, aquatic animals carry nearly their entire muscle tissue mercury burden in the methylated form. These observations, of course, apply only to organisms growing in near-natural environments; those growing in waters highly contaminated with other specific mercury species, especially organomercurials, may show bioaccumulation of these species as well.

The previously reported lower and more variable methylmercury percentages in fish may have many causes, which vary in importance with both time and laboratory. The most significant of these are probably sample handling prior to analysis and analytical procedures which tend to err on the low side for methylmercury and on the high side for total mercury. These problems may have been more prevalent in the past, when the analytical methods were first being developed and there was less awareness of the potential for contamination in handling.

In addition, the handling of the data, once generated, can have a strong influence on the final results which are reported. Although this author has seen no evidence of published data where high percent methylmercury results were reduced to 100%, the practice has apparently been employed in regulatory and monitoring reports. In this study, we showed that the combined effect of two factors, uncontrolled sample handling procedures and rounding down of high percent methylmercury values, reduced the mean percent methylmercury for the data set from 98.5 to 90.4% (Table 3).

Also of significance to the overall data set is what is done with "anomolous" data points. In this study, about 5% of the original methylmercury – total mercury pairs differed by greater than 35% (with almost all cases showing low methylmercury results). These data points, upon being run in triplicate, were usually found to be errant and the correct, much closer to 100% methylmercury values substituted into the data set. If only the original data were used, the overall mean percent methylmercury for the data set would have been reduced by an additional 3%. In the past, where less knowledge existed to suggest uniformity in percent methylmercury levels, there would have been little reason to reanalyze such samples. The fact that it is also quite costly to do so probably reduces the level of reanalysis on most monitoring projects.

That the mercury content of fish muscle tissue is nearly all in the methylated form has important geochemical as well as regulatory implications. Geochemically, this observation, combined with recent measurements of mercury speciation in water (Bloom et al. 1991), allows the calculation of bioconcentration factors for methylmercury of 10^6–10^7, while those for nonmethylmercury are seen to be $<10^4$. Thus, the overwhelmingly important questions in assessing anthropogenic contributions to fish mercury levels are those relating to the processes affecting methylation of mercury in the environment and its uptake by organisms.

On the regulatory side, quite clearly, only one standard is needed for mercury levels in fish tissues, and this may be assessed using total mercury rather than methylmercury monitoring. This is significant from a logistical standpoint; since far more laboratories can accurately measure total mercury in tissues than methylmercury and since the cost of such analysis may be only 10–20% of what it costs for methylmercury determination, many more fish could be more reliably monitored if the regulations were based solely on total mercury content.

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The data presented in this paper were assembled from a variety of monitoring and research projects over the past 2 yr. The following organizations have agreed to share data, collected for other purposes, to make this paper possible: Electric Power Research Institute (Palo Alto, CA), CH2M Hill (for USEPA) (Bellevue, WA), CDR Consultants (for Allied-Signal) (Stowe, MA), Tetra-tech, Inc. (for USEPA) (Lafayette, CA), and Dairyland Power (LaCrosse, WI). In addition, I would like to thank Ms. Ann W. Grant for the analysis of many of the samples reported here and Ms. Sharon K. Goldblatt for her editorial expertise.

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Back-Calculation of Fish Lengths Based on Proportionality between Scale and Length Increments

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The assumption underlying linear back-calculation of fish lengths (L) from scale measurements (S) is that annual increments of length (ΔL) are proportional to annual increments between the annuli on scales (ΔS) for each fish–scale combination. A sample from a fish population that provides measurements of fish length and scale radius at time of collection should be symmetrical transversely to its central axis when plotted with an absolute slope of 45°; if not, it can be made symmetrical by either of two easy methods. The slope of the central axis can be estimated using either an arithmetic mean regression or (preferably) the geometric mean regression; either should be made to pass through the centroid of the whole sample. *Average* lengths at each age are unbiased if computed from average annulus distances using one of the above symmetrical statistics whereas biased estimates are obtained using either of the ordinary regressions between S and L, especially when part of the range of ages (older than 0) is missing at either end of the sample. Lengths of individual fish can be back-calculated throughout their life by using either the Whitney–Carlander or (preferably) the Fraser–Lee procedure, with the fixed parameter estimated from a symmetrical regression line.

Selon l'hypothèse sous-jacente au calcul à rebours de la longueur des poissons (L) à partir de valeurs mesurées des écailles (S), les accroissements annuels de longueur (ΔL) sont proportionnels aux accroissements annuels des distances entre les anneaux des écailles (ΔS) de chaque combinaison poisson–écaille. Un échantillon d'une population de poissons à partir duquel on a mesuré la longueur des poissons et le rayon des écailles au moment du prélèvement devrait présenter une symétrie transverse par rapport à son axe central lorsque reporté en utilisant une pente absolue de 45°, mais si ce n'est pas le cas, deux méthodes d'application facile permettent d'obtenir la symétrie. La pente de l'axe central peut être estimée à partir d'une régression de moyennes arithmétiques ou, de préférence, de moyennes géométriques, les deux pentes devant passer par le centroïde de l'ensemble de l'échantillon. Les longueurs *moyennes* de chaque âge ne sont pas biaisées si elles sont déterminées à partir des distances moyennes entre les anneaux en utilisant l'une des méthodes statistiques symétriques ci-dessus, mais elles sont biaisées si l'une des régressions habituelles entre S et L est utilisée, surtout lorsqu'une partie de la gamme des âges (plus que 0) est absente à l'une ou l'autre des extrémités de l'échantillon. Les longueurs de poissons donnés peuvent être calculées à rebours pour tout moment de leur vie en appliquant la procédure Whitney–Carlander ou, de préférence, celle de Fraser–Lee, le paramètre fixe étant estimé à partir d'une droite de régression symétrique.

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1. Introduction

The recent paper by Francis (1990) contains a valuable review of the literature concerning the calculation of fish lengths at successive ages from marks on scales, otoliths, etc., which should be read by everyone in the field. However, his conclusions require modification in one important respect. This paper will show that the point of origin of proportional back-calculations should either be determined biologically or be located on a central axis that is symmetrical with respect to fish length and scale radius, computed from a sample that is symmetrical transversely to that axis when plotted with an absolute slope of 45°.

The argument will be in terms of radial distances measured on a scale from the central focus to successive annuli and to the scale margin, which is the measurement usually used for ctenoid scales. However, the treatment applies equally to such other scale measurements as may be appropriate for individual species. It will not necessarily apply to growth *during* any growing season, almost certainly not if this includes more than one "stanza" of development (Ricker 1979), although this rarely occurs at ages beyond 0, certain anadromous fishes being the principal exception.

Terms and abbreviations to be used are as follows:

L = length of a fish,
$\bar{L}$ = average length of an age-group or a population
ΔL = an increment of length during 1 yr
S = radius of a scale, measured from focus to margin
$\bar{S}$ = average radius of scales used for age determination
ΔS = an increase in radius during 1 yr, measured between successive annuli on a scale
FSC = a fish–scale combination used in back-calculation
C-sample = an assemblage of FSCs that constitute a sample taken from a fish population for the purpose of back-calculation; also called, when plotted, an array of (L_C, S_C) observations
Centroid = the point described by the mean of L and S in a C-sample, or in any portion of it
L_C = length of a fish in a C-sample

S_C = radius of a scale from the above fish
L_i = length of a fish at age i
S_i = radius of a scale at age i
OR = an ordinary least-squares regression line, or its slope
GMR = the geometric mean regression line, or its slope; also called the standardized, or reduced, major axis (of an L–S array)
AMR = see Appendix 2
K = the intercept of an L–S regression line on the L-axis
Q = the intercept of an L–S regression line on the S-axis.

Symbols and concepts to be used are as follows, where L and S are measured from their means:

Σ = summation symbol
N = number of FSCs in an age-group or in a whole C-sample
b = OR of L on S: $\Sigma LS/\Sigma S^2$
d = OR of S on L: $\Sigma LS/\Sigma L^2$
r = coefficient of correlation between L and S: $(bd)^{1/2}$
s_L = standard deviation of L: $[\Sigma L^2/(N-1)]^{1/2}$
s_S = standard deviation of S: $[\Sigma S^2/(N-1)]^{1/2}$
v = GMR of L on S: $[\Sigma L^2/\Sigma S^2]^{1/2} = (b/d)^{1/2} = s_L/s_S$
$1/v$ = GMR of S on L.

2. The Basis of Linear Back-Calculation

Back-calculations of fish length (L) from scale annuli (S) are made from a sample of fish taken from a population, here called a "C-sample." The necessary characteristics of such a sample are discussed in Section 4. The basic premise to be used is that which is stated or implied in most if not all linear back-calculations, namely, that after the first annulus is formed, for each fish–scale combination (FSC) the annual increment of $S(\Delta S)$ is directly proportional to the annual increment of $L(\Delta L)$. This of course implies that there will be a linear relation between L and S. However, L will not necessarily be proportional to S. In other words, a line relating L and S need not pass through the origin of the graph. Usually it will intersect the L-axis at a positive value of L; call this K.

If we were dealing with only one fish and one scale that were measured in successive years, obviously $\Delta L/\Delta S$ would be the slope of the line relating L and S. Two or more FSCs can differ in two ways: their L–S relationships can have different slopes, or different K-values, or both. Among those that have the same $\Delta L/\Delta S$ but different K-values, the L–S relationships (*trajectories* of Campana 1990) will of course all have the same slope (Table 1; lines 2–4 of Fig. 1). For FSCs that have the same K-value but differ in $\Delta L/\Delta S$, the L–S trajectories radiate from the point K on the L-axis (Fig. 2). Such slope differences are a result of differences in scale size at a given fish length, either because the scales are from different positions on a fish's body or from fish-to-fish variability even at one position. FSCs can of course differ both in slope and in the position of the intercept K (Fig. 1).

Back-calculation of the size of each fish from its own L–S relationship is not practical, and indeed would be pointless: if we know L and S for each age, there is nothing to calculate. However, what is available is not the magnitude of L and S for each FSC at successive ages, but only the magnitude of L and S at capture (L_C, S_C) for fish of as many ages as are available in the C-sample. If the sampled fish were all taken outside of the growing season, so that the edge of the scale marks the position of the final year's annulus, then the average linear relation between $\bar{L}_C$ and $\bar{S}_C$ over several ages can be used to represent the average linear relation between $\bar{L}_i$ and $\bar{S}_i$ in the population. If the sample is taken during the growing season, but all at one time, then the $\bar{L}_C$–$\bar{S}_C$ relation can still be used, although with slightly less assurance (Section 11c).

In order to obtain a trajectory from which to calculate L from S at all ages, it is only necessary to locate the best value for L when $S = 0$. Because this point cannot be located for each FSC separately, an overall average value must be used. Two types of estimate have been employed: biological and mathematical. Fraser (1916) examined young chinook salmon (*Oncorhynchus tshawytscha*) and found that the average length at which a scale was first formed (more exactly, the length at which its focus was first established) was 2 in. (50.8 mm). This he considered to be the value of $\bar{L}$ when $\bar{S} = 0$, so he made proportional calculations from that point. This implies that $\Delta L/\Delta S$ has the same value *within* age 0 as it has *between* later ages, but there is practically no possibility of significant back-calculation error from failure of this assumption (cf. Section 8). Recently, Campana (1990) has proposed using a biological origin for back-calculating from otoliths, which differ from scales in that they are usually already formed when a fish hatches. Thus, his bio-

TABLE 1. Patterns of increase in length of fish and scale over 4 yr, with means and standard deviations (s). Rows 1–5 compare three FSCs having the same $\Delta L/\Delta S$ but different values of L and S at age 1, with their variability in length increasing from age 1 to age 2 and decreasing from age 3 to age 4. Rows 6–10 compare FSCs having different values of $\Delta L/\Delta S$ but the same length increments throughout life: they could be three different scales from the same fish. L = fish length (cm); S = scale radius (mm).

	Age 1		Age 2		Age 3		Age 4	
$\Delta L/\Delta S$	L	S	L	S	L	S	L	S
10	20	0.5	35	2.0	55	4.0	70	5.5
10	30	1.0	50	3.0	70	5.0	80	6.0
10	40	1.5	70	4.5	90	6.5	95	7.0
Mean	30	1.0	51.67	3.167	71.67	5.167	81.67	6.167
s	10	0.5	17.56	1.258	17.56	1.258	12.58	0.764
40	25	0.5	45	1.0	65	1.5	75	1.75
20	25	1.0	45	2.0	65	3.0	75	3.5
10	25	2.0	45	4.0	65	6.0	75	7.0
Mean	25	1.167	45	2.333	65	3.500	75	4.083
s	0	0.764	0	1.528	0	2.291	0	2.673

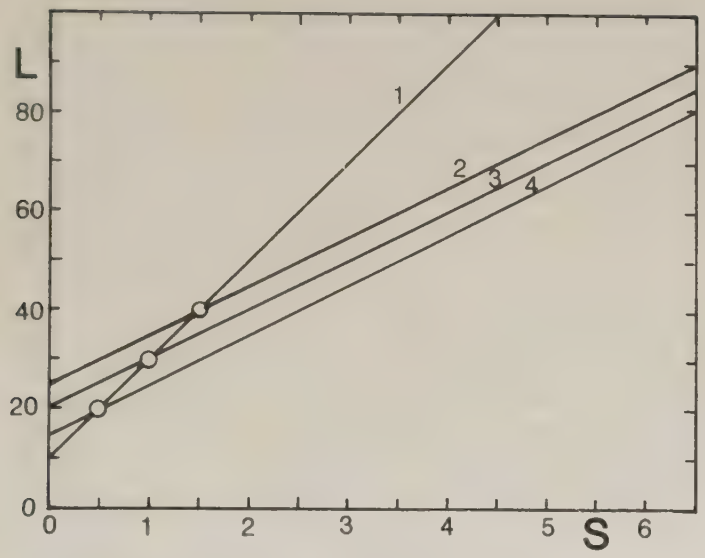

FIG. 1. Diagram of L–S trajectories that pass through three different values of (L_1, S_1), shown as open circles. Lines 2–4 all have $\Delta L/\Delta S = 10$ and meet the L-axis at three different points: $K = 25$, 20, and 15. Line 1 has $\Delta L/\Delta S = 20$; it happens to pass through all three values of (L_1, S_1) and meets the L-axis at $K = 10$. L = length (cm); S = scale radius (mm).

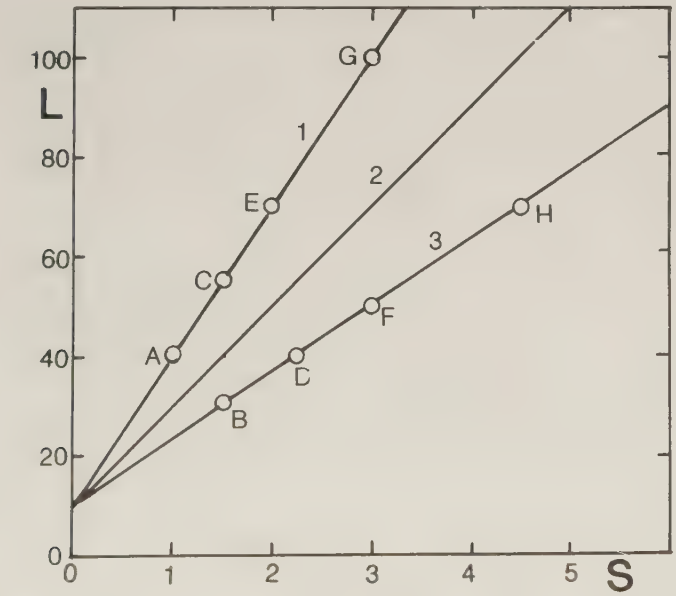

FIG. 2. Diagram of three L–S trajectories, all having a common point of origin ($L = 10$, $S = 0$) but different values of $\Delta L/\Delta S$, as follows: 1, 30; 2, 20; 3, 13.33. Line 2 has an absolute slope of 45°. See Appendix 2 regarding points A–H. Units as in Fig. 1.

logical origin is "the fish and otolith length corresponding to the initiation of proportionality between fish and otolith growth. In many species this point would occur at the time of hatch."

In many investigations, fish of age 0 are not available for study, so these biological methods cannot be used. Lee (1920) was the first to estimate an origin for back-calculation trajectories by extrapolating a linear relation between L_C and S_C back to its intercept on the L-axis. The problem here is, what line should be used? Lee used the ordinary regression (OR) of L_C on S_C, and most later writers have done the same. However, because there is no reason to consider either L or S as dependent on the other, the necessary characteristic of any line that describes the average relation between the two is that it should be *symmetrical* with respect to L and S, in the sense that if L and S are interchanged, the estimated slope is replaced by its reciprocal. Only if this is true will the line pass through or close to the points $(\bar{L}_i, \bar{S}_i)$, the average value of L and of S for each age-group represented. And because we need an average value of L at $\bar{S} = 0$ on which to anchor the individual $\Delta L/\Delta S$ trajectories, only a symmetrical axis will be able to provide what is required.

Two types of estimate of this symmetrical axis are available. One is the geometric mean regression (GMR), which is the ratio of the standard deviations of L and S, that is, a measure of the vertical dispersion of the observations divided by their horizontal dispersion (or vice versa, depending on how the coordinate axes are labelled). A second type of symmetrical estimates of the central axis of a sample are the arithmetic mean statistics (AMRs) described in Appendix 2.

3. Frequency Distributions of Fish Length and Scale Radius

Experience with numerous populations of centrachid, percid, and salmonid fishes has indicated that most of their length distributions are reasonably symmetrical about a central value at each age. Examples are shown for bluegill (*Lepomis macrochirus*) in fig. 3.2 of Ricker (1975) and for yellow perch (*Perca flavescens*) in table 2 of Hile and Jobes (1941); the latter have a much greater dispersion relative to the mean because they include fish taken at different seasons and in different years. In any event, a normal (Gaussian) distribution makes a convenient model for illustration.

Consider then a C-sample taken from all the ages in a fish population. Typically it will consist of a series of near-normal length distributions, most or all of them overlapping. If values of L in an age-group are normally distributed or nearly so, values of S will have a similar shape. If the correlation between L and S were perfect, the points on a graph of L against S would all lie on a single straight line; but because the correlation is always less than perfect, what we get is a series of overlapping elliptical or near-elliptical arrays of observations, one for each age.

The usual pattern for growth in length of a year-class of fish is an increse in variability during the first few years and later a decrease in variability as the initially slow-growing individuals tend to catch up. If all the FSCs in a year-class had the same value of $\Delta L/\Delta S$, the size of the L–S ellipses would first increase and later decrease, following the above trends (upper five rows of Table 1). However, when scales of different sizes are used so that $\Delta L/\Delta S$ varies between FSCs, the ellipses become larger with age. The lower five rows of Table 1 show that an increase in the variability of S would then occur even if all the fish increased in length at the same rate. A similar schedule would show that the fish would increase in length variability even when FSCs are compared whose scales all have the same pattern of increase in radius.

A mortality rate, either natural or from fishing, that varies with size within any age-group of a year-class can also change the shape of an L–S array. However, this may have less effect than one might suppose: Jones (1958) showed that a linear type of size-selective mortality gradient does not change a normal distribution of length in an age-group, or even reduce its standard deviation, although it does shift the mean (illustrated in table 2 and fig. 2 of Ricker 1969).

If the value of $\Delta L/\Delta S$ is independent of the length of each FSC within an age-group, then the major axes of the L–S ellipses for each age will all conform to a single straight line, within limits of sampling error. If, however, $\Delta L/\Delta S$ is correlated with L (and with S) within an age-group, such alignment will not occur. Specifically, if $\Delta L/\Delta S$ is smaller among the slow-growing fish, the slope of the major axis at each age will be less than that of the overall axis that joins the centroids of successive ages. Conversely, if slow-growing fish tend to have relatively larger scales, then the within-age major axes will have greater slopes than the overall axis. The latter situation has been found for the *otoliths* of a number of marine species, in studies cited by Campana (1990), but I know of no similar studies for scales.

In any event the (L_C, S_C) observations in a C-sample form an elongate band. This can be quite narrow if L and S are closely related (r = 0.96–0.99). This can occur when "key scales" are used, taken from a fixed position on a fish's body (see, for example, fig. 1 of Lindroth 1963), and the range of lengths present is large. If, however, the size of the scales used and hence $\Delta L/\Delta S$ varies considerably, the band of (L_C, S_C) observations will be broader, on the whole, and will probably increase in breadth from age 1 onward.

4. Characteristics of a Good C-Sample

Whereas we want back-calculated lengths to apply to the whole of a fish population, the regression line used in computations has to come from the C-sample taken from that population. Although one or more different segments of a population may sometimes be sampled randomly, using different kinds of apparatus, it is impossible to obtain a random sample of an entire population in the wild, even when age 0 is excluded (as is desirable because they have not yet formed an annulus). Moreover, the relative number of fish of different ages in a population is continually changing. Apart from that, a random sample of an entire population older than age 0 would not be the best C-sample, for fish of age 1 or 2 are sometimes numerous enough to have disproportionate influence. On the other hand, we would not want the sample to include equal numbers of fish of every age because this would give too much weight to the larger ones.

The best solution appears to be to aim at a more or less equal number of L–S observations at equal intervals of length, but it is not important that this rule be followed closely if a symmetrical axis is used for back-calculation and the C-sample is or has been made symmetrical about that axis when at 45° absolute slope (Section 5).

What should be avoided, if possible, is including in the sample fish taken at different times during the growing season. Such mixtures would, strictly speaking, require that $\Delta L/\Delta S$ be constant not only from year to year but also within the year whereas Francis (1990) cited several studies in which small deviations from within-year constancy were observed. But often, of course, an investigator has little choice and must use whatever scales come from routine sampling of sport or commercial catches. A fairly common type is shown in Francis's fig. 1, where there is a concentration of points at either end of the array and fewer in between (Appendix 1).

Although C-samples cannot be random samples of a whole population, it is essential that they be reasonably close to symmetrical transversely to their own (and the population's) central axis when this axis is plotted with an absolute slope of 45°. The reason for this stems from a basic characteristic of an array of points. Most of the lines that have been fitted to an L_C–S_C array maintain their position, relative to the observations, regardless of the units used for L and S and regardless of the scale intervals chosen for plotting. Examples are the two ORs, the GMR, and the AMRs. These can be called *conformable* lines. However, lines drawn at right angles to a conformable line are *not* conformable. If such a line is used to divide an array into two halves, the identity of the observations in the two divisions will differ, depending on what absolute slope that line may happen to have when plotted (cf. fig. 2 and 3 of Ricker 1984). Thus, a standard slope for the axis is necessary, and the only possible choice for this role is 45°. At this absolute angle the GMR coincides with another well-known (but nonconformable) line, the ordinary major axis or apparent trend of an array. Thus, at 45° the GMR is an estimate of the central axis of a symmetrical array, both in fact and in appearance.

5. Samples Truncated by Length

If a C-sample is truncated at or in the region of a particular length by the nature of the sampling apparatus, the *apparent* extent of the truncation will vary with the absolute slope of any conformable trend line plotted, and at some slopes even the existence of truncation may not be recognizable. When the C-sample is plotted with a slope close to 45°, it is possible to recognize reasonably accurately, by eye, the region affected by truncation and to reject all observations in this region. This may be done either by rejecting the whole of the age-group(s) affected or by drawing a line transversely to the axis of the *unaffected* region when it is plotted at 45° absolute slope.

In general, it is not easy to find measurement units or graph paper intervals that give a set of L_C–S_C points a slope really close to 45°, but a less than perfect line can be used for a preliminary assessment and removal of any obviously asymmetrical points, after which a new line can be fitted and a new assessment made. If an exact diagram of the points with a 45° axis is desired, the observations of L and S can each be divided by its own standard deviation, in which case the plotted axis will have an absolute slope of 45° and a numerical value of 1. Presumably a computer could be programmed to do this work with dispatch.

Once assured that the L–S array is, or has been made, symmetrical, the observations in their original units can be used to estimate either the GMR or an AMR because both are "conformable" statistics.

If two or more sampling methods have contributed to a C-sample, as is usual, there can be truncation by length in the middle of the sample as well as at the ends. However, truncation in this region has much less effect on the overall slope of regression lines than does truncation at the extremes and can almost always be ignored. Moreover, truncation at the upper end of the lower of two component samples will tend to be compensated by truncation at the lower end of the upper one, assuming both types of truncation are present.

6. Which Regression?

The regression line that has most frequently been used to establish an intercept for back-calculation is not either of the symmetrical lines, but is the OR of L on S. The reason is, presumably, that for a bivariate normal C-sample, it provides the best estimates of L from S *in that sample*. But C-samples are not often close to bivariate normal, and when they are, it

reflects the type of sampling done rather than the structure of the population sampled. Thus, an OR does not estimate a property of a fish population in the way that symmetrical statistics do.

To illustrate, consider a C-sample with observations reasonably symmetrical transversely to the 45°axis of the array and fairly evenly distributed along that axis. Such a C-sample's axis passes through or close to each age's average length *in the population* whereas the ORs do not. Specifically, the OR of L on S gives estimates of $\bar{L}_i$ that are too large at scale sizes less than the mean of the C-sample ($\bar{S}$) and too small when S is larger than $\bar{S}$. The error increases with distance from $\bar{S}$ in both directions, although it will not usually be very large even at the extremes if all ages are included in the sample.

Note that when the OR of L on S is used instead of a symmetrical statistic, the average annual *increase* in length will be overestimated for age 0, but at all later ages it will be too small, simply because the slope of an OR is always less than that of the array's central axis.

The bias of an OR is more serious when the distribution of observations in the C-sample is not at all uniform over the range of fish and scale sizes in the population. For example, a fairly common deficiency of C-samples is failure to include one or more of the younger annulus-bearing ages. In that case the OR of L and S may give estimates of $\bar{L}_i$ with only a small amount of bias in the ages sampled, but they can become grossly inaccurate when the line is extrapolated to unsampled ages. If observations at the younger ages become available and are added to the sample, the centroid is shifted toward them and the slope of the OR becomes greater, both of which changes will decrease the estimates of $\bar{L}_i$ at the previously missing ages.

More generally, if older fish predominate in a sample, the centroid will be situated close to them and the two OR lines will be far apart at the younger ages. If younger ages predominate, the centroid shifts to smaller sizes, and the two ORs come closer together at the younger ages and are farther apart at the older ones. *The symmetrical regressions, however, are not changed by such moves, except for random shifts resulting from the improved accuracy provided by each increase in size of the total sample.*

Carlander (1982, his fig. 1) shows the distribution of published values of the intercept K that have been estimated from the OR of L on S by various authors for six centrarchid and one percid species. These typically range from 10 to 50 or 60 mm, with a few even more extreme values. The situation seemed so unlikely and unsatisfactory that Carlander proposed using a standard value of K (his a) for each species (his table 1). These are averages of the estimates that he considered to be most reliable, and he noted that if GMRs had been used, they would have been smaller. Actually, even using the ORs, smaller K-values would have been obtained if all the samples had contained fish of sizes down to the smallest of age 1, so that the slopes of the ORs would be steeper and the centroids of the arrays would be shifted toward the L-axis, both of which reduce the estimate of K. Elimination of any artificial truncation by length that may have been present at either end of the arrays would also reduce K-estimates made from either an OR of L on S or from the GMR. Thus, Carlander's excellent initiative could probably be improved by reducing the standard values of K, perhaps by 10–20 mm for different species. However, it seems best to wait for a few new or revised estimates of K for each species, based on symmetrical L_C–S_C axes of symmetrical C-samples.

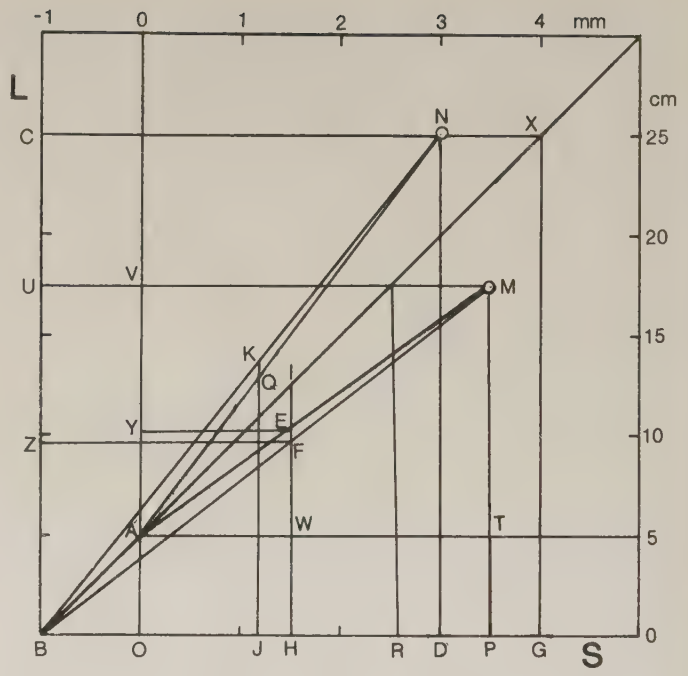

FIG. 3. Illustration of back-calculations of lengths from scales (M and N) that lie on either side of a symmetrical relationship (line BAX) between fish length (L) and scale radius (S).

7. Back-Calculation of Mean Lengths of Age Groups

The principal object of most back-calculations is to estimate the *average* length of each age-group in a sample at the successive ages indicated on the scales. These can be computed from average annulus measurements using a symmetrical axis directly. The expression for computation of average lengths is

$$(1) \quad \bar{L}_i = K + v\bar{S}_i$$

where v is the slope of the axis of the symmetrical C-sample, estimated by the GM or AM method, K is that axis's intercept on the L-coordinate axis, $\bar{S}_i$ is the mean of the annulus measurements at age i, and $\bar{L}_i$ is the age-i average length to be estimated from $\bar{S}_i$.

8. Back-Calculation of Length for Individual Fish

Back-calculations for individual fish are usually done along $\Delta L/\Delta S$ trajectories that join each (L_C, S_C) point to an origin where $S = 0$. The value of L when $S = 0$ cannot be estimated for each FSC individually, so an average value must be used. This has been obtained either by direct measurement on age-0 fish or, more often, by extrapolating a line fitted to a series of (L_C, S_C) observations (Section 2).

For example, in Fig. 3, line BAX represents such a (symmetrical) fitted line, and OA is the average value of L when $\bar{S} = 0$. Point M represents a scale whose radius (OP) is greater than the age-group average radius (OR) for a fish of its length (OV) and OH is the distance from its focus to the first annulus. Then because line AEM is a diagonal of both of the similar rectangles $AYEW$ and $AVMT$, $AY/AW = AV/AT$. Changing to the symbols used up to now, and generalizing to any age i, this becomes

$$(2) \quad \frac{L_i - K}{S_i} = \frac{L_C - K}{S_C}$$

where S_C and L_C are scale and fish size at capture, K is the L-axis intercept of a line relating L_C and S_C in a C-sample, and L_i is the fish length to be estimated from a scale annulus at S_i. This method of back-calculation can be called the Fraser–Lee method, using that name in a broad sense to describe any computation whose parameter is an L-axis intercept K, regardless of how the position of K is determined. Some authors seem to restrict the term Fraser–Lee to computations which determine K from the OR of L_C on S_C, but this is inappropriate because Fraser (1916) used a biological method to estimate K whereas Lee (1920) was the first to use a mathematical method, although she too made some reference to observations on age-0 herrings.

An alternative, although rarely used, method of computing individual lengths, proposed by Whitney and Carlander (1956), uses the intercept of an average L–S relation on the S-axis as the point from which all the $\Delta L/\Delta S$ trajectories radiate. For example, in Fig. 3 the line BFM is a common diagonal of rectangles $BZFH$ and $BUMP$, so that $BZ/BH = BU/BP$. Bearing in mind that OB is negative, and generalizing to any age i, this becomes the expression

$$(3)\quad \frac{L_i}{S_i - Q} = \frac{L_C}{S_C - Q}$$

where Q is the S-axis intercept of a line relating L_C and S_C, and the other symbols are as for expression (2). The term Whitney–Carlander method also is used here in a broad sense to describe all expressions whose parameter is an S-axis intercept.

In the Fraser–Lee computation, each scale radius is adjusted to the average radius for a fish of its length; hence it has been called a "scale-proportional" or SPH method by Francis (1990). The Whitney–Carlander method he calls "body-proportional" or BPH; in it, each fish length is adjusted to the average length of fish that have the radius measurement of the scale being used. He also suggested that the SPH method requires that the L–S relationship used to estimate K be the OR of S on L rather than the commonly used OR of L on S but that the BPH method should use the OR of L on S to estimate Q. On this basis the two methods tend to give quite different computed values of L_i, especially at the younger ages (Appendix 1). Francis associates the two methods with two different hypotheses but can suggest no criterion for choosing between the two, which leaves everyone in an uncomfortable quandary.

The thesis of this paper is that, whatever their statistical credentials, neither of the ORs is appropriate for establishing an average point from which proportional back-calculations are to be made. The reason is simply the the ORs diverge increasingly from the mean value of fish length and scale radius ($\bar{L}_i$, $\bar{S}_i$) at the youngest ages; hence, extrapolating them into the age-0 region and beyond cannot possibly estimate either $\bar{L}$ when $\bar{S} = 0$ or $\bar{S}$ when $\bar{L} = 0$. Instead, the same symmetrical axis should be used for both the Fraser–Lee and the Whitney–Carlander methods.

However, there is still the problem of choosing between the two methods, or for that matter, choosing some position on the L–S axis other than K or Q as origin for proportional back-calculations. In the latter event, a computation similar to expression (2) or (3) would require two parameters:

$$(4)\quad \frac{L_i - L_p}{S_i - S_p} = \frac{L_C - L_p}{S_C - S_p}$$

where (L_p, S_p) are the coordinates of the point on the C-sample's central axis that has been chosen to be the origin for proportional back-calculations. (Campana's (1990) similar expression (4) gives the same result, but when rearranged in the form and with the symbols of expression (4) here, it has L_C and S_C instead of L_p and S_p on the left-hand side.)

Fortunately, for practical purposes it is not important to know exactly where the best average point of origin for the individual $\Delta L/\Delta S$ lines is located on the C-sample's central axis. This can be illustrated by comparing the calculation made by the Fraser–Lee and Whitney–Carlander methods in Fig. 3, using two FSCs whose $\Delta L/\Delta S$ trajectories deviate rather widely from the central axis. For the FSC at point M the age-1 computed length using the Fraser–Lee method is HE whereas using the Whitney–Carlander method it is HF, the difference being EF, about 6 mm. Similarly for point N, for the first annulus at J the Fraser-Lee estimate is JQ and the Whitney–Carlander estimate is JK, again a difference of 6 mm. These can be considered unlikely maximum differences, not only because M and N are distant from the axis, but also because $K = 5$ cm and $Q = -1$ mm are rather extreme values for a species of the size indicated. (Compare $K = 1.90$ cm and $Q = -0.341$ mm for the herring of Appendix 1.) The differences at older ages are all less than 6 mm because the $\Delta L/\Delta S$ trajectories converge on points M and N. Also, the differences have opposite signs on either side of the central axis, so that *there is no average difference at all between the two types of calculation* as long as the same symmetrical central axis is used to obtain the intercepts for both.

We may conclude that either the Whitney–Carlander or the Fraser–Lee method will usually or always provide usable back-calculated lengths. However, Fraser–Lee is preferable because K describes, approximately at least, an objective point in the development of the fish: the beginning of scale growth, whereas Q implies a group of fish of zero average length!

9. Random Sampling Variability

As far as I know, there has been no statistical examination of distributions resembling the overlapping ellipse-like arrays suggested above. If from a C-sample, computations of L for individual fish are made using expression (2) or (3), the variances of these estimates at each age can be computed directly, those from expression (3) being slightly larger than those from expression (2). However, in both cases there is additional variability that comes from the sampling error of the position of the C-sample's GMR or AMR line.

The variance of a GMR is the same as that of the corresponding OR, which can be computed by standard methods. Hence, a minimum estimate of the variance of a point on a GMR could be obtained, for orientation, by using the standard formula for the L-variance of a point on the C-sample's OR of L on S. When correlation is large, a GMR and the corresponding OR are not far apart; hence the L-variance of a point on the GMR at a given value of S should be similar to, but somewhat greater than, that of the corresponding point on the OR. Specifically, the L-variance of the point $(\bar{S}_i, \bar{L}_i)$ can then be added to the age-i variance of L that was computed as in the preceding paragraph.

Even the best possible estimates of the variances of computed L_i and $\bar{L}_i$, when they become available, will not express the whole of the uncertainty in back-calculations. Another source of error, which may be either random or biased, is the misreading of ages. In duplicate tests, different readers rarely if ever agree completely about any long series. Also, the GMR or AMR that is used may be somewhat biased in relation to that of the population, in spite of the precautions and adjustments

discussed earlier. These possibilities make it all the more important to avoid introducing a known source of systematic error by using the wrong line in back-calculations. This is particularly important if L and S are not closely correlated, and C-samples with correlations less than 0.9, or even less than 0.8, have in fact been used.

10. Lee's Phenomenon

Lee's phenomenon (LP) of "apparent change in growth rate" is a tendency for back-calculated lengths at any age to be smaller, the larger the fish from which they were calculated. It was reported for sprats by Sund (1911) and for herring, haddock, and trout by Lee (1912). Both authors had made their back-calculations assuming direct proportionality between L and S. Later, Lee (1920) showed that part of what they observed was a computational artifact. When lengths are calculated by direct proportion, so that K of expression (2) is put equal to 0 when in fact it has a positive value, back-calculated lengths automatically become smaller, the larger the fish from which they are calculated. More generally, LP occurs whenever K is assigned a value less than the best one, estimated biologically or from a symmetrical regression line. Conversely, if K is assigned a value greater than the above, it produces "inverse LP," a tendency for calculated lengths to be larger, the larger the fish from which they are calculated.

Much the commonest departure from best back-calculation procedure has been putting K equal to the L-axis intercept of the OR of L_C on S_C instead of to the (always smaller) intercept of a symmetrical regression line. By itself, this produces *inverse* LP. In spite of this, *direct* LP is of very frequent and widespread occurrence among published back-calculations, while inverse LP is uncommon. This indicates that there is some other mechanism causing direct LP, usually powerful enough to reverse the effect of using too large a value of K. Lee (1920) suggested that this could be the effect outlined by Lea (1913), based on successive annual recruitments, of the larger of the still unrecruited members of a year-class, to the mature schools that were sampled. A more generally applicable explanation is the one proposed by Sund (1911) that the faster-growing members of a year-class have a greater mortality rate (natural or from fishing) than slow-growing ones, during part of their life at least. It is also possible for a stock to exhibit inverse LP in early life, when the smaller fish are more vulnerable to predation, and direct LP later (Ricker 1975, section 9.4.1).

11. Discussion

(a) Campana (1990, his fig. 3) showed that when faster-growing fish have smaller otoliths (T) at any given length (L) within an age-group, any line describing the trend of (L, T) observations for that group will deviate from the average value of $\Delta L/\Delta T$; hence, it would not be a suitable line for establishing a point of origin for proportional back-calculations. Campana was interested especially in the growth of fish during their first year of life, for which this effect could be important, so he proposed using the biological point of origin described in Section 2 above. It has yet to be shown that fast-growing fish have relatively smaller (or larger) *scales*, but even for otoliths the effect will be very much less important when an axis is fitted to (L_C, T_C) observations for several ages, as compared with a single age. And if the axis is fitted to the *means* of the (L_C, T_C) observations for each age, there should be no bias at all (Appendix 2).

(b) We of course wish all back-calculated lengths to refer to a standard time of year: the period between the formation of an annulus and the start of the next year's growth. If the scales available were taken *during* a growing season, it becomes necessary to know or to assume that $\Delta L/\Delta S$ is constant throughout that interval, although small deviations from within-age linearity have been described in several papers cited by Francis (1990). However, trial calculations suggest that, for practical purposes, the assumption of within-age uniformity of $\Delta L/\Delta S$ will almost always be satisfactory. Only part of the final year's increment in length is affected for each FSC; for all earlier years the complete year's growth is obtained from the annulus-to-annulus distance.

(c) As defined here, ΔL and ΔS are increments of length and of scale radius during 1 yr. This needs to be modified slightly because it has been observed, for some populations at least, that growth of a scale beyond an annulus begins somewhat later in the growing season as a fish becomes older. Thus, an annulus-to-annulus year may be longer than a calendar year by up to a week or so. This effect emphasizes the need for caution in determining ages during the early part of the growing season, especially among the larger fish.

(d) The development of a fish from the fertilized egg onward can be divided into several stages of *stanzas* that are separated by some abrupt change in morphology or rate of growth. The transition between stanzas may be either severe (as in eels) or merely a change in the relative growth rate of body parts (Ricker 1979). The latter kind of change happens in some species of salmonid fishes when they enter salt water. If this occurs during or at the end of the first year of growth, as in many *Oncorhynchus*, it is not a problem for back-calculations that go back only as far as the first annulus. However, there are species or stocks that spend 2 or more yr in freshwater before going to sea, for example many *O. nerka* and most stocks of *Salmo salar*. For the latter, Lindroth (1963, his fig. 2–4) showed that scale measurements, plotted against fish length, have a greater slope during their freshwater life at 40–200 mm than they have later in the ocean. Hence, back-calculations would have to be made separately for these two stanzas.

(e) If all age-groups in a population were to be randomly sampled at the end of a growing season, and if the survival of fish in an age-group is not related to their size, then $\bar{L}_C$ should be an estimate of $\bar{L}_i$ at each age i, in which event it would not be necessary to make back-calculations. Thus the reasons that back-calculations are needed are three: (1) most samples are taken within a growing season, so back-calculation is needed to estimate length at the end of the last complete year of growth; (2) *random* samples of all ages in a population are rarely available; in particular, samples from the younger ages often include more of the larger individuals of those ages, and sometimes one or more ages are completely absent; (3) growth rate and survival rate frequently are correlated, usually negatively, in which event the mean back-calculated size at a given age is smaller, the greater the age of the fish from which it was calculated ("natural" LP, assuming unbiased back-calculation procedure). The correlation may have a physiological or ecological cause, or be a result of selective fishing, or any combination of these. Hence, back-calculated sizes should be separated on the basis of the total age of each FSC, in order to determine whether natural LP is present. If it is, then the problem of decid-

ing what is to be an average growth rate at each age is difficult, but must be faced (Ricker 1969, 1979). Under these conditions, comprehensive expressions like the Gompertz or Pütter (Brody–Bertalanffy) curves, fitted to the observed lengths of the survivors at each age, will indicate growth that is slower than the actual from age 1 onward.

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Appendix 1. Statistics from the Herring Example of Lee (1920) and Francis (1990)

Lee's appendix table II includes 200 North Sea herring. Francis plotted these as an L–S array in his fig. 1, and his regression equations for them are (1) OR of L on S: $L = 2.93 + 52.4S$ and (2) OR of S on L: $S = -0.0134 + 0.0169L$ (both L and S are in centimetres). The centroid of the array is where these lines cross at $\bar{S} = 0.3156$, $\bar{L} = 19.47$, found by solving the simultaneous equations. The correlation between L and S is $(52.4 \times 0.0169)^{1/2} = 0.941$, and the GMR of L on S is $(52.4/0.0169)^{1/2} = 55.68$. Passing a line of this slope through the centroid gives the GMR equation for L on S as $L = 1.897 + 55.68S$ and that for S on L as $S = -0.03407 + 0.01796L$, two expressions for the same line.

The observations used in the herring back-calculation are $L_C = 28.5$ cm, $S_C = 0.41$ cm, $S_1 = 0.17$ cm. Using the Fraser–Lee or SPH method with the OR of S on L, so that K of expression (2) is $0.0134/0.0169 = 0.793$, Francis (p. 892) computed $L_1 = 12.28$ cm; using Whitney–Carlander or BPH with the OR of L on S, so that Q of expression (3) is $-2.93/52.4 = -0.0559$, he obtained $L_1 = 13.82$ cm, a difference of 1.54 cm.

Using the GMR equation above, the L-axis intercept is $K = 1.897$; hence, from expression (2), $L_1 = 12.93$ cm. Similarly the S-axis intercept of the GMR line is $Q = -0.03407$; and from expression (3), $L_1 = 13.10$ cm. Thus the difference between these Whitney–Carlander and Fraser–Lee computations, using the GMR line, is only 0.17 cm at age 1 and would be progressively smaller at older ages. Also, the difference would be negative for any scale from that fish that was larger than 0.48 cm, which is the average scale size computed from the GMR line using $L_C = 28.5$ cm.

It may be worth noting that if the two ORs were to be used in the traditional manner, i.e. opposite to Francis's recommendation, the difference between their computed values of L_1 is reduced, but not by much. In that case, for SPH, $K = 2.93$ and $L_1 = 13.53$ cm and for BPH, $Q = -0.0134$ and $L_1 = 12.35$ cm. The difference is 1.18 cm instead of 1.54, and the SPH value is of course now larger than the BPH.

Appendix 2. Comparison of Arithmetic Mean and Geometric Mean Regression Lines

Seeking a central trend line or axis that would be symmetrical with respect to Y and X variates, Wald (1940) proposed dividing an array of Y–X observations into two halves and joining their centroids. A little later, K. R. Nair and colleagues (e.g. Nair and Banerjee 1942) proposed dividing the array into three parts and joining the centroids of the two outer portions. Bartlett (1949) adopted Nair's proposal and improved it slightly by passing a line of Nair's slope through the centroid of the entire array. All of these procedures were shown to be consistent estimates of the same central axis, the Nair–Bartlett procedure being somewhat more efficient, at least for large samples. It is also true that any other symmetrical division of a symmetrical array, with or without omission of a section of it, will produce a consistent, although less efficient, estimate of the same central trend line (Wald 1940).

However, as orginally described, these AM methods all overlooked a practical difficulty. In order to be symmetrical, they must divide an array transversely to a central axis plotted at 45° absolute slope, which axis's position has yet to be established. To meet this, a trial division can be used first, its position corrected by successive iterations; usually, one is sufficient. Another problem is that with large arrays there can be difficulty in deciding on which side of a division line a particular observation lies.

These problems can be avoided if an array is naturally subdivided into segments, which may even overlap somewhat. Because (L_C, S_C) observations in a C-sample are structured by age, a simple way to apply the Wald method is to combine the

ages into two groups having numbers of observations as nearly equal as possible and then join their centroids to obtain an estimate of the symmetrical slope of the array. With this procedure, it is not necessary that the individual ages be symmetrical about the C-sample's 45° axis, although they may well be so; it is only necessary that their means lie on that axis, within limits of sampling variability. Hence, this method could be used if there is appreciable deviation from overall symmetry of the C-sample caused not by selective sampling, but by the existence of a correlation between $\Delta L/\Delta S$ and L within age-groups, or because of an effect of a length-related mortality rate. All AMRs are of course conformable lines, in the sense of Section 4.

The GMR too is conformable and symmetrical. In addition, it is analogous to a least squares estimate in that it minimizes the sum of the areas (all considered positive) of the triangles formed by projecting each observation horizontally and vertically onto the line. Also, if a sample is perfectly symmetrical (i.e. for each point on one side of the 45° axis there is a point opposite and at the same absolute distance from that axis on the other side) the GMR and the AMRs estimate the same line exactly.

To illustrate this, in Fig. 2 the two pairs of symmetrical points *AB* and *CD* have a centroid at ($\bar{L} = 41.25$, $\bar{S} = 1.5625$); similarly, *EF* and *GH* have a centroid at ($\bar{L} = 72.5$, $\bar{S} = 3.125$). Thus the Wald estimate of the slope of their central axis is

$$(72.5 - 41.25)/(3.125 - 1.5625) = 20.$$

At the same time the GMR of all the points *A–H* is

$$s_L/s_S = 22.510/1.1255 = 20.$$

In practice the exact symmetry described above never occurs. We can only make sure that an array *appears* symmetrical transversely to its central axis when that axis is plotted with an absolute slope of 45°. Thus, there are always small differences between AM and GM estimates of a central axis. I prefer the GMR because of its simple form (the ratio of two standard deviations), because it coincides with the ordinary major axis at 45°, because of its similarity to a least squares estimate, and because there is no need to decide how an array should be divided up.

Influence of Stream Flows and Stock Size on Recruitment of Arctic Grayling (*Thymallus arcticus*) in the Chena River, Alaska

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Clark, R. A. 1992. Influence of stream flows and stock size on recruitment of Arctic grayling (*Thymallus arcticus*) in the Chena River, Alaska. Can. J. Fish. Aquat. Sci. 49: 1027–1034.

The hypothesis that recruitment of Arctic grayling (*Thymallus arcticus*) in the Chena River is influenced by stream flows and stock size was tested using population data collected from 1976 through 1990. Recruitment may be influenced by stream flows during the initial weeks of life of Arctic grayling, namely during spawning, emergence, and the larval stage. Using correlation and regression analyses, stream flow during the time-frame was found to be a significant descriptor of variability in recruitment ($r = -0.751$, $P = 0.005$). Although stream flows were implicated in recruitment variation, creation of an environment-dependent, stock–recruitment model was not possible because estimates of measurement error were lacking, because of bias due to the relation between residuals and subsequent stock size, and because of the presumed autocorrelation of stock size. An alternative analysis was conducted to investigate the influence of stock size on recruitment when stream flows were thought to minimally affect recruitment. Using an estimate of natural mortality rate and assuming no fishing mortality, the theoretical contribution of recruits to the spawning stock exceeded the maximum observed stock size. I concluded that the maximum observed stock size failed to negatively influence recruitment, and the level of stock size that might influence recruitment is greater than the maximum observed stock size.

À l'aide de données démographiques sur l'ombre arctique (*Thymallus arcticus*) recueillies dans la rivière Chena de 1976 à 1990, l'auteur a vérifié l'hypothèse que le débit et les effectifs influent sur le recrutement. Il se peut que le débit influe sur le recrutement au cours des premières semaines du cycle vital de l'ombre arctique, soit lors de la fraie, de l'émergence et du stade larvaire. À l'aide d'analyses de corrélation et de régression, l'auteur a établit que le débit au cours de la période expérimentale était un important descripteur de la variabilité du recrutement ($r = -0{,}751$, $P = 0{,}005$). Il n'a pu toutefois élaborer un modèle du recrutement au stock lié à des variables environnementales étant donné l'absence d'estimations de l'erreur d'échantillonnage, le biais lié à la relation entre les résidus et les effectifs suivants, ainsi que l'autocorrélation supposée des effectifs. Il a ensuite effectué une autre analyse pour déterminer l'influence des effectifs sur le recrutement lorsque le débit n'avait qu'une faible influence prévue sur celui-ci. En se basant sur un taux estimatif de mortalité naturelle et en supposant une absence de mortalité par pêche, il a déterminé que la contribution théorique de recrues au stock reproducteur dépassait la taille maximum observée du stock. Il a donc formulé la conclusion que cette dernière n'avait pas d'influence négative sur le recrutement et qu'elle était inférieure au niveau des effectifs qui pourrait influer sur le recrutement.

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Limited research has shown that stream flows may be an important factor influencing year-class strength of freshwater and anadromous fish. For example, Crecco and Savoy (1984) found that June stream flows negatively affected recruitment of American shad (*Alosa sapidissima*) in the Connecticut River. High June stream flows interfered with feeding success of first-feeding shad larvae, causing high mortality (Crecco and Savoy 1987). Harvey (1987) found that spates in one Oklahoma stream killed larvae of minnows (central stone roller (*Campostoma anomalum*), and bigeye shiner (*Notropis boops*)) and sunfish (green sunfish (*Lepomis cyanellus*) and longear sunfish (*L. megalotis*)) smaller than 10 mm total length. Downstream displacement (and death) of these species decreased as these species grew to lengths greater than 10 mm total length. Trout species are also susceptible to high river flows. Elwood and Waters (1969) found that two year-classes of brook trout (*Salvelinus fontinalis*) were virtually eliminated by two separate flood events in Valley Creek, Minnesota. Seegrist and Gard (1972) reasoned that winter floods destroyed eggs of the fall-spawning brook trout and May floods destroyed eggs of the spring-spawning rainbow trout (*Oncorhynchus mykiss*). Alternatively, brown trout (*Salmo trutta*) recruitment may be positively correlated with April stream flows in chalk-streams (Soloman and Paterson 1980). Higher flows were hypothesized to reduce competitive interactions among age 0 brown trout, resulting in higher numbers per reach. Abundance of young striped bass (*Morone saxatilis*) was positively correlated with the amount of river flow into the estuary during June and July (Turner and Chadwick 1972). Increased river flows may have delayed the time of spawning and caused young bass to be distributed further downstream, enhancing growth and survival. Central to these studies is the finding that stream flow events during a critical phase of early life history had a profound effect on recruitment.

Recruitment of Arctic grayling (*Thymallus arcticus*) may also be influenced by stream flows. For example, Vincent (1962) surmised that logging practices in Michigan during the early 1900's caused artificial freshets, destabilizing spawning habitat for Arctic grayling. These changes in habitat from logging and overexploitation in fisheries were presumed to cause the extinc-

tion of Arctic grayling in Michigan (Vincent 1962). Based on the early life history of Arctic grayling and the apparent susceptibility of eggs and larvae to high stream flows, I investigated the hypothesis that stream flows during the known periods of egg deposition, hatching and emergence, and larval stage could influence year-class strength in the Chena River. Alternatively, if stream flows did influence recruitment of Arctic grayling, then did the number of spawners also influence recruitment? No recruitment can occur when no spawners are present, and in reality, the abundance of spawners in the Chena River is greater than zero. However, experience tells us that detection of the influence of stock size on recruitment is generally difficult (Goodyear and Christensen 1984) and fraught with bias when quantified (Walters and Ludwig 1981; Walters 1985). Although faced with these obstacles, I attempted to detect the influence of parent stock on recruitment of Arctic grayling and add an additional reference point (other than the obvious point at the origin) to the underlying stock–recruitment relation.

Methods

Much of the information concerning early life history of Arctic grayling in Alaska is not in the published literature but has been summarized from agency progress reports and environmental consultant reports by Armstrong (1986). Characteristics of the early life history of Arctic grayling are central to the development of a stream flow based approach to investigating variability in recruitment. Inclusion of unpublished material in the analysis was necessary due to the newness of Arctic grayling research in this area.

Data Sources

The literature contains qualitative data on the reproductive habits and early life history of Arctic grayling that can be applied to the Chena River. Spawning occurs between mid-May and mid-June in Alaska (summarized by Armstrong 1986), with spawning in the Chena River occurring from mid-May (Reed 1964; Walker 1983) through early June. Spawning coincides with water temperatures of 4°C. Arctic grayling reach sexual maturity at 270 mm fork length, corresponding to an average age of 5 yr (summarized by Armstrong 1986). Hatching time varies due to temperature, averaging from 16 to 21 d at temperatures of 8–10°C (Rawson 1950; Watling and Brown 1955). Kratt and Smith (1977) found that the alevins remained under gravel for 3–4 d. Newly emerged larvae average 13 mm total length (Sytina 1964; Walker 1983). Larval Arctic grayling may be vulnerable to high stream flows for up to 2 wk after emergence (Nelson 1954). Lee (1985) found that larval Arctic grayling have poorly developed fins, which persists until the juvenile stage, and prefer the low current velocities found at the margins of riffles and in slow side pools. Amur grayling (*Thymallus arcticus grubei*) and Arctic grayling have also been observed along quiet stream margins and side pools during high water, but were unable to return to the main channel after water levels receded (Syttina 1964 and Lee 1985, respectively). Lee (1985) also found that juvenile Arctic grayling seek deeper water after fin development has completed.

In contrast with the general lack of quantitative data on spawning and early life history, extensive stock data exist for juvenile and adult Arctic grayling in the Chena River. Stock assessments have been performed annually since 1967, with

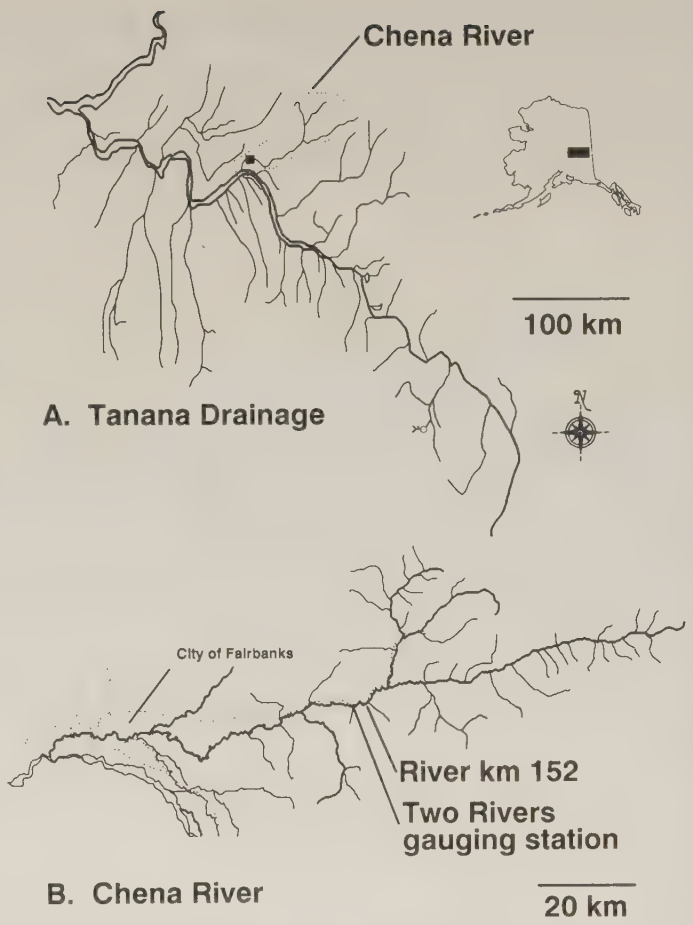

FIG. 1. (A) Tanana River drainage of interior Alaska; (B) Chena River drainage with the location of the Two Rivers gauging station and upstream extent of population assessments of Arctic grayling.

quantitative estimates of abundance and age composition for 1976 through 1990. Parent stock (S) and recruitment (R) data were collected in mark–recapture experiments on fish age 3 and older in the lower 152 km of the river (Fig. 1) with the use of a boat-mounted electrofishing unit. These experiments were conducted in July of each year. From 1976 to 1985 the average number of Arctic grayling was estimated in three to five 4.8-km sections of the river (Clark 1990). Population size for the lower 152 km of river was estimated by expanding these averages by the number of river kilometres that represented similar habitat and hydrologic conditions to each section along the lower 152 km of river (Fig. 1). From 1986 through 1990, population size was estimated in mark–recapture experiments encompassing the lower 152 km of river (Clark 1990). Ages were determined from scales taken during the mark–recapture experiments. Lacking data on lengths of fish marked and recaptured, abundance, and age composition were not adjusted for length selectivity of the electrofishing gear for stock assessments in 1976 through 1984. However, these adjustments were made for data collected by electrofishing from 1985 through 1990. Similarly, standard errors were calculated for abundances and age compositions during 1985 through 1990, but were not available from 1976 through 1984.

For this study, recruitment was defined as the abundance of age 3 Arctic grayling in the lower 152 km of the Chena River. Historically, the recreational fishery harvests fish greater than 150 mm fork length, or approximately age 3. Parent stock was defined as the abundance of age 5 and older Arctic grayling in

TABLE 1. Estimates of recruitment, stock size, and standard errors for Arctic grayling in the lower 152 km of the Chena River, and average stream flow during 16 May through 24 June of 1976 through 1987 (data sources: Clark 1990, 1991; USGS 1977–88).

Year-class t	Year of recruitment $t + 3$	Recruits (age 3) R_{t+3}	SE$[R_{t+3}]$	Stock size (age 5 and older) S_t	SE$[S_t]$	Stream flow 16 May – 24 June ($m^3 \cdot s^{-1}$) D_t
1976	1979	18 525		33 345		31.1
1977	1980	18 525		13 110		49.8
1978	1981	24 510		9 025		14.8
1979	1982	5 415		5 795		40.6
1980	1983	24 415		10 735		15.2
1981	1984	9 025		17 290		24.0
1982	1985	9 595	2 095	16 150		64.7
1983	1986	23 750	10 418	15 010		21.7
1984	1987	2 755	358	15 200		61.7
1985	1988	3 373	529	37 145	24 950	82.6
1986	1989	4 332	491	17 612	7 718	34.7
1987	1990	16 881	4 172	8 316	1 088	28.0
Average		13 425		16 561		39.1
SD		8 581		9 525		21.5
CV		63.9		57.5		55.0

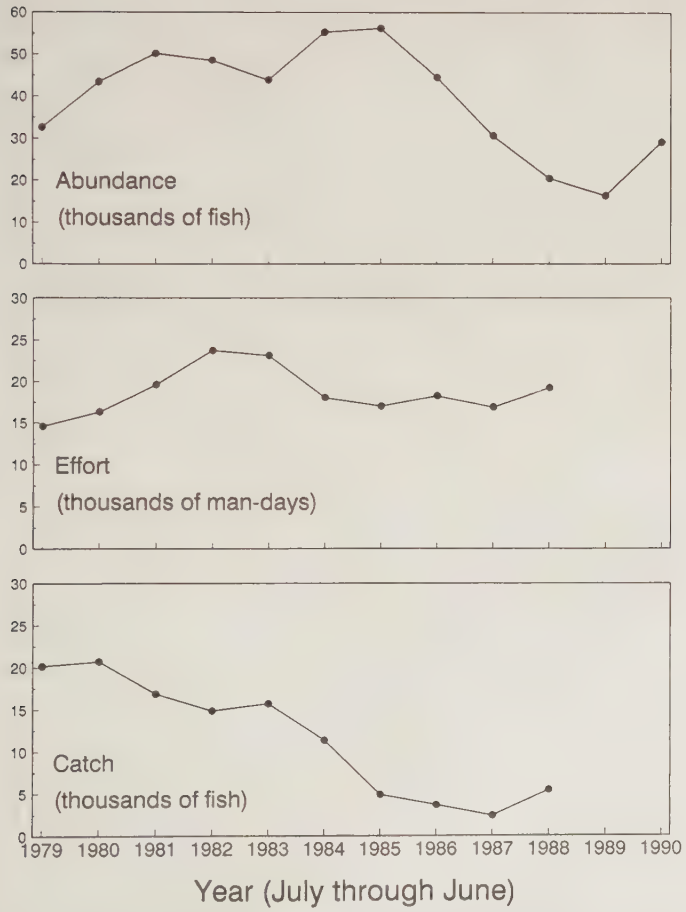

FIG. 2. Estimates of Arctic grayling abundance, recreational fishing effort, and recreational catch in the Chena River for statistical years (July through June) 1979 through 1990.

the same section of river. Consequentially, there is a 3-yr lag between egg deposition and recruitment and a 2-yr lag between recruitment and contribution to the spawning stock (Table 1).

Catch and effort data were also collected on an annual basis through a statewide angler survey by Mills (1990). These data were reported for each calendar year between 1979 and 1989 and they pertained to the entire Chena River drainage. However, stock assessment data are applicable only for the lower 152 km of the mainstem Chena River and germane to the month of July. To make these two sets of data compatible, catch and effort data were recalculated to reflect catch and effort from the lower 152 km of river after the mark–recapture experiment had concluded. To recalculate, I assumed that harvest of Arctic grayling occurred during the months of May through October and that 219 km of the Chena River was readily accessible to anglers. Additionally, the fishery was closed during the month of May from 1987 through 1990. Catch (and effort) made after the mark–recapture experiment in year t was estimated by

$$(1) \quad C^* = (0.5C_t + 0.5C_{t+1}) \frac{152 \text{ km}}{219 \text{ km}}$$

for the years without a fishery closure in May (1979–86) and

$$(1.1) \quad C^* = (0.6C_t + 0.4C_{t+1}) \frac{152 \text{ km}}{219 \text{ km}}$$

for the years with a fishery closure in May (1987–89), where C_t is the catch (or effort) in year t and C_{t+1} is the catch (or effort) in year $t + 1$. As a result, estimates of catch and effort are missing for 1989 (July 1989 through June 1990) because estimates for January through June of 1990 have not yet been calculated. While fishing effort has remained relatively stable during the period of this study, annual catches have decreased dramatically (Fig. 2). Moreover, before 1987, fishery regulations consisted of a five-fish daily bag and no size limit. Since 1987, additional fishery regulations have been imposed, consisting of a mimimum size limit (305 mm total length) and a closure of the fishery during the spawning period. The daily bag limit was subsequently reduced from five per day to two per day in 1990.

Influence of Stream Flows

To assess the hypothesis that stream flow affects subsequent recruitment during the period from egg deposition to the end of the larval stage, daily stream flows were averaged for several

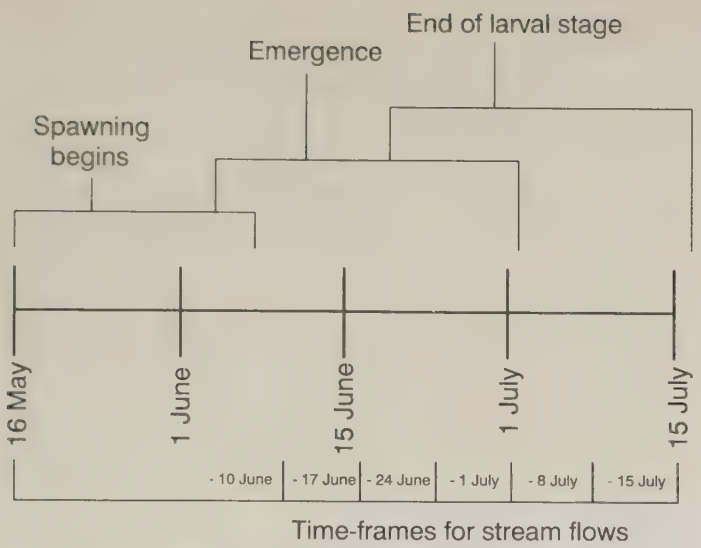

FIG. 3. Time sequence of spawning and the early life history of Arctic grayling, and time-frames for stream flow used in the analysis.

"time-frames" encompassing the published times of spawning, hatching and emergence, and duration of the larval stage. The earliest date of observed spawning was 15 May, so that stream flow might begin to affect survival on 16 May. Hatching occurs 16–21 d after spawning, resulting in emergence dates ranging from 4 June until 10 June.

Assuming the larval stage lasts 2 wk, the end of the vulnerable period ranges from 20 June through 24 June. Therefore, the time-frame chosen to represent flows from the known period of spawning through the end of the larval stage was set to 16 May through 24 June (Fig. 3). However, spawning may be delayed until early June in some years, so that the hypothesized period of vulnerability to stream flows could extend into the middle of July (Fig. 3). To investigate the sensitivity of the model to possible changes in spawning dates and emergence times, five other time-frames for stream flows were constructed. These other time-frames for stream flow all started on 16 May and terminated on 10 June, 17 June, 1 July, 8 July, and 15 July. Stream flow data were collected on a daily basis at a United States Geological Survey (1977–88) monitoring station located at river kilometre 147 (Fig. 1) and converted from cubic feet per second to cubic metres per second.

The model chosen to link stream flow to recruitment variability was

$$(2)\quad R_{t+3} = \alpha S_t \exp(-\varphi D_t)\exp(-\epsilon_t)$$

where R_{t+3} is the abundance of age 3 recruits in year $t + 3$, S_t is the parent stock in year t, D_t is the average stream flow during the time-frame in year t, α and φ are parameters to be estimated, and ϵ_t is a multiplicative error term as suggested by Ricker (1975). This model treats stream flow and any other unexplained factor as a series of multiplicative survival rates. The logarithmic transform was used to linearize equation 2:

$$(3)\quad \log(R_{t+3}/S_t) = \log(\alpha) - \varphi D_t - \epsilon_t$$

where R_{t+3}, S_t, D_t, α, φ, and ϵ_t are as described above. Moreover, the logarithmic transformation was necessary to normalize the dependent variable, since estimates of abundance from mark–recapture experiments are lognormal. Correlation between $\log(R_{t+3}/S_t)$ and D_t in the context of equation 3 is a measure of how important stream flow during the time-frame has been to year-class strength in the last 12 yr (Fig. 4). Linear regression and analysis of residuals could then be used to assess the appropriateness of the model. Any departure from conditions necessary to linear regression (heteroscedasticity, autocorrelation of residuals, or undue influence by one or more residual) would invalidate the model (Draper and Smith 1981; Abraham and Ledolter 1983). Stream flow was deemed appropriate as an independent variable, since stream flow was not highly correlated with parent stock (Pearson's $r = 0.485, P = 0.110$) and the time series of average stream flows was not significantly autocorrelated across years (Table 2).

Influence of Stock Size

Assuming that there is a significant relation between stream flows and subsequent recruitment, why would not the removal of this component of variability allow the development of a traditional stock–recruitment model? Assuming that the residuals from equation 2 are related to parent stock, a model could be developed:

$$(4)\quad R_{t+3} = \alpha S_t \exp(-\varphi D_t - \beta S_t)\exp(-\epsilon_t)$$

where β is the density-dependent parameter to be estimated. However, the standard nonlinear regression recipe for this model would be

$$(5)\quad R_{t+3} = \alpha S_t \exp(-\varphi D_t - \beta S_t) + \epsilon_t.$$

Unfortunately, equation 5 suffers from three major shortfalls. First, the error term is not multiplicative, and thus violates the accepted theory of survival rates. Secondly, without a logarithmic transform or the ability to perform weighted regression, any violation of assumptions necessary to regression analysis would invalidate the model. As noted above, abundance estimates are lognormally distributed, so that a logarithmic transform is needed. Lastly, and most importantly, equation 5 will produce biased estimates of parameters if S_t is related to ϵ_t (Walters 1985). Iteroparous fishes, such as Arctic grayling, are prime examples of this relation, since there is a 2-yr interval before ϵ_t becomes part of S_{t+2}. The problem can be exacerbated by regulations prohibiting harvest of Arctic grayling until they are age 5 (or 305 mm fork length), forcing S_{t+2} to relate directly to R_t if natural mortality rate is constant. This problem tends to inflate estimates of β (Walters 1985).

A logarithmic transform would solve some of the problems inherent in equation 5. However, several other problems arise as a result of the ratio $\log(R_{t+3}/S_t)$ regressed on D_t and particularly S_t. The coefficient of variation of average recruitment is similar to the coefficient of variation of average parent stock (Table 1), so that correlation between $\log(R/S)$ and S could be totally dependent on the variation in S and not on R as is intended. The result regression would "beg the question" of a relation between S and R, since the relation of S to the residuals from a regression analysis of equation 3 could be a result of the transformation and not the result of a functional relation.

With these important considerations in mind, I examined the stock–recruitment data for year-classes that might indicate a potential for density dependence when effects due to stream flows would be minimal. Since density-dependent mortality theoretically occurs at the highest levels of parent stock, I looked only at year-classes produced from high stock sizes. Two data points have high values for parent stock, the 1976 and 1985 year-classes (Table 1). Recruitment from the 1985 spawning event may have been negatively influenced by high

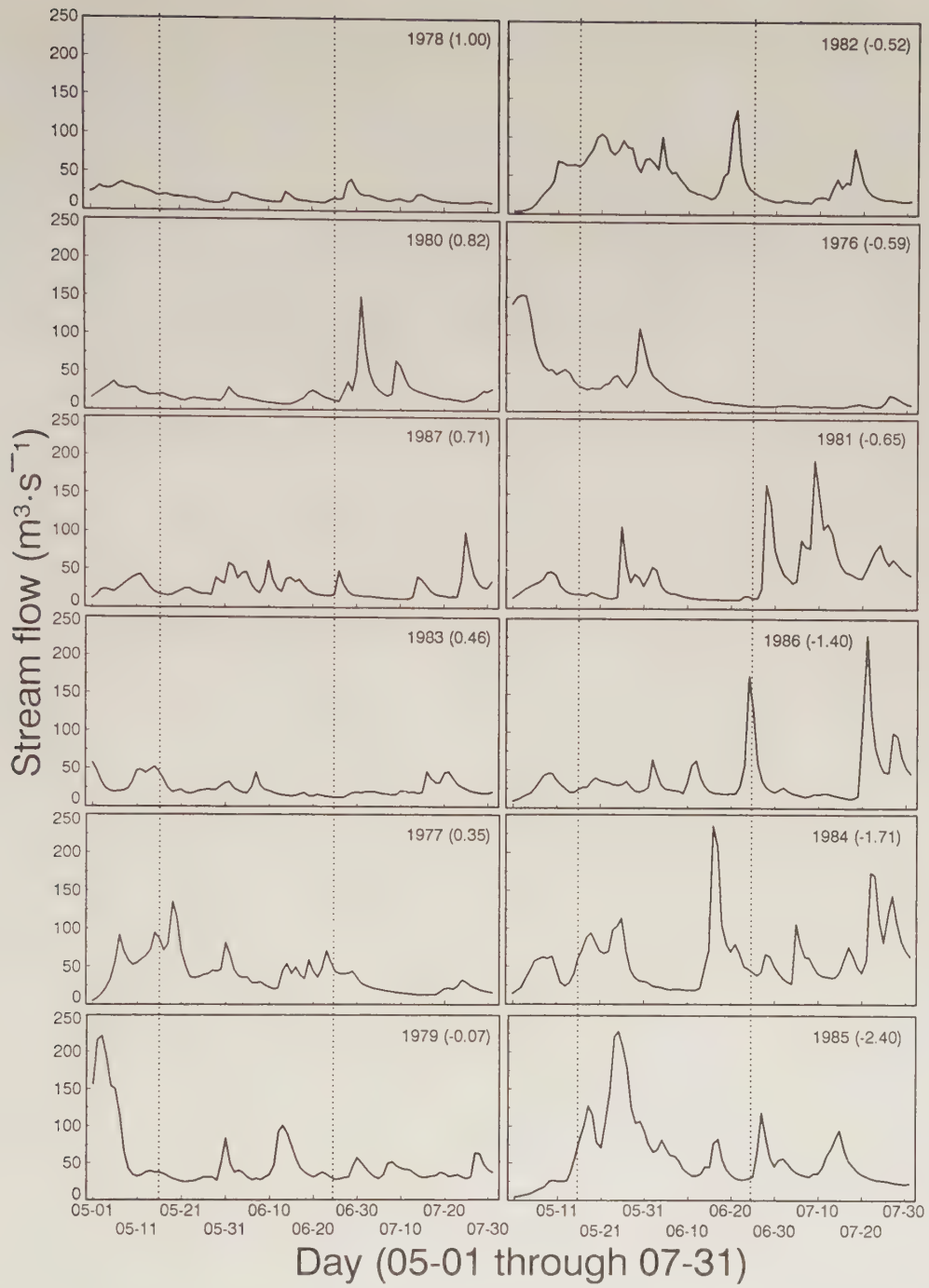

FIG. 4. Daily stream flows during 1 May through 31 July at the Two Rivers gauging station (river kilometre 147) from 1976 through 1987. Panels of daily flows by year are arranged in descending order by $\log(R_{t+3}/S_t)$ (in parentheses). Dotted lines delimit the 16 May through 24 June time-frame for stream flows.

TABLE 2. Sample autocorrelations and standard errors for the first four lags of average stream flow (D_t) during 16 May through 24 June of 1976 through 1987.

Lag k	r_k	SE
1	−0.087	0.289
2	−0.067	0.291
3	0.227	0.292
4	−0.342	0.306

stream flows and would not represent a point on the underlying stock–recruitment relation. However, the 1976 year-class may not have been influenced as heavily by stream flow as in 1985. To investigate where the 1976 year-class might lie on the stock–recruitment curve, I needed to estimate the average instantaneous mortality rates for the stock during 1979 through 1987. Using the average instantaneous natural mortality rate, I reasoned that if the conditions present in 1976 were to persist indefinitely (without fishing mortality) and density-dependent mortality was not influencing recruitment, then the observed recruitment would subsequently replace or exceed the observed

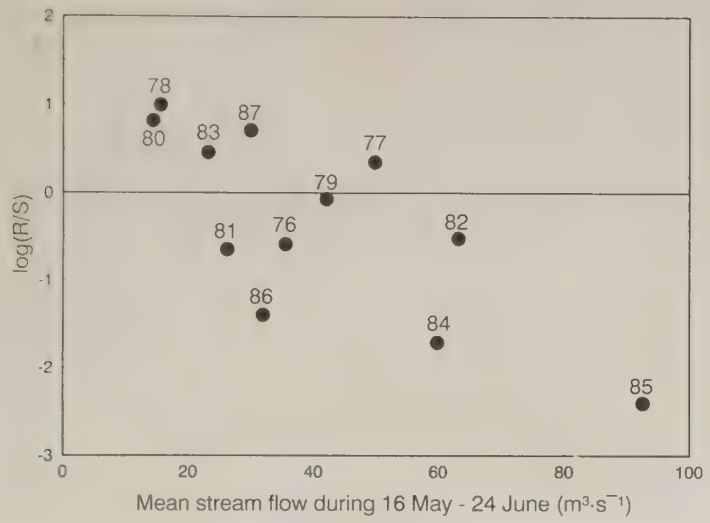

FIG. 5. Plot of $\log(R_{t+3}/S_t)$ against mean stream flow (16 May through 24 June) for the 1976 through 1987 year-classes.

parent stock on an annual basis. In this context, replacement would occur when the cumulative contribution of recruits to the spawning stock equals or exceeds the number of spawners that produced these recruits. Obviously, replacement can occur at any constant level of recruitment, but I wished to see if replacement occurs during conditions of high stock size and low stream flows. Alternatively, if density-dependent mortality was influencing recruitment, then the parent stock in 1976 would not be replaced. While not a definitive test of density dependence, this procedure will identify the relative position of the 1976 parent stock to the theoretical level of parent stock at which density dependence occurs.

To estimate natural mortality rate, I first estimated total mortality rate by cohort for the 1979 through 1987 stocks:

$$(6)\quad Z_t = -\log\left(\frac{N_{t+1,6+}}{N_{t,5+}}\right)$$

where Z_t is the instantaneous rate of total mortality for year t, $N_{t+1,6+}$ is the abundance of age 6 and older Arctic grayling in year $t+1$, and $N_{t,5+}$ is the abundance of age 5 and older Arctic grayling in year t. The average instantaneous rate of fishing mortality was then estimated with the Baranov catch equation, solving for $\bar{F}$ (Ricker 1975):

$$(7)\quad \bar{F} = \frac{\bar{C}\,\bar{Z}}{\bar{N}\,(1-\exp(-\bar{Z}))}$$

where $\bar{F}$ is the average instantaneous rate of fishing mortality, $\bar{C}$ is the average catch in the lower 152 km of river, $\bar{N}$ is the average abundance (age 3 and older), and $\bar{Z}$ is the average instantaneous total mortality rate for the years 1979 through 1988. Assuming constant effort (Fig. 2) and therefore constant instantaneous natural mortality rate:

$$(8)\quad \bar{M} = \bar{Z} - \bar{F}$$

where $\bar{M}$ is the average instantaneous natural mortality rate.

To see if the parent stock of 33 145 fish observed in 1976 could be continually replaced by an annual recruitment of 18 525 fish, recruitment was discounted by the average natural mortality rate and the resulting abundances at age summed:

$$(9)\quad S = \sum_{t=2}^{u} R\exp(-\bar{M}t)$$

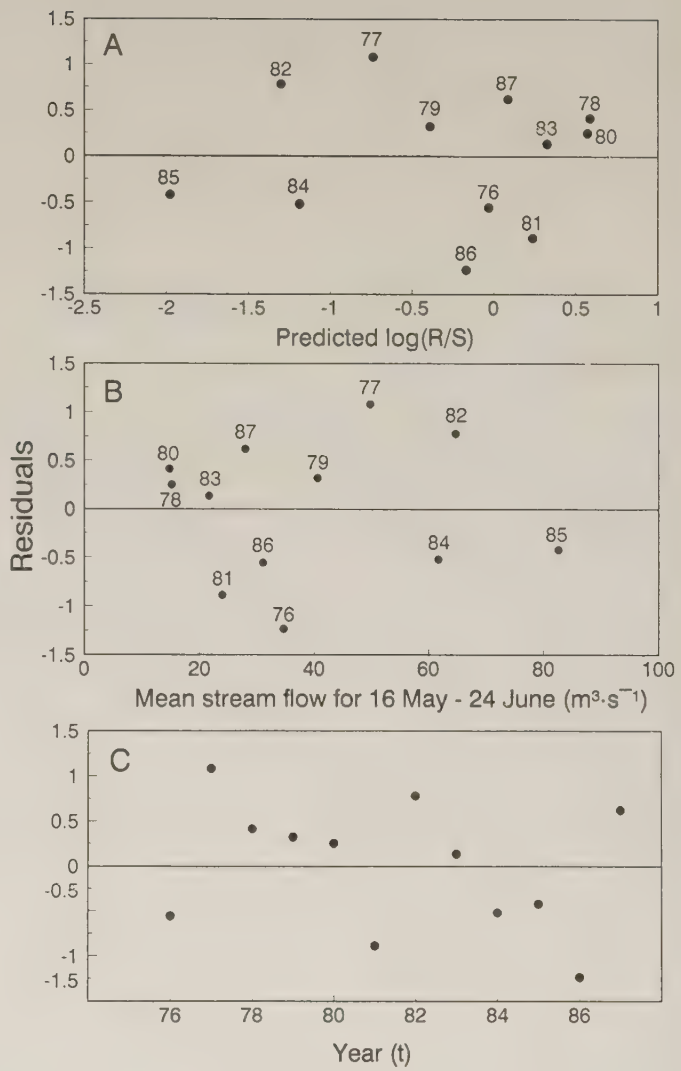

FIG. 6. (A) Plot of residuals against predicted values of $\log(R_{t+3}/S_t)$ for the 1976 through 1987 year-classes; (B) Plot of residuals against mean stream flows (16 May through 24 June) for the 1976 through 1987 year-classes; (C) Plot of residuals against year.

TABLE 3. Correlations, regression coefficients, coefficients of determination (r^2), and tail probability (P) for the relation between $\log(R_{t+3}/S_t)$ and six different time-frames of stream flow.

Flow time-frame	r	Regression coefficients: Intercept	Slope	r^2 [a]	$P\ (F > F_0)$
16 May – 10 June	−0.718	0.935	−0.030	0.467	0.008
16 May – 17 June	−0.756	1.111	−0.036	0.529	0.004
16 May – 24 June	−0.751	1.144	−0.038	0.521	0.005
16 May – 01 July	−0.787	1.371	−0.044	0.582	0.002
16 May – 08 July	−0.803	1.500	−0.049	0.609	0.002
16 May – 15 July	−0.809	1.576	−0.051	0.619	0.001

[a] r^2 adjusted for degrees of freedom.

where S is the predicted parent stock from an annual recruitment of R (18 525 fish in this case), $t = 2, 3, 4,\ldots,u$ years after recruitment, and u was set to 12 (corresponding to an age of 15 yr).

TABLE 4. Autocorrelations and standard errors for the first four lags of residuals from the regression of $\log(R_{t+3}/S_t)$ on average stream flow (D_t) during 16 May through 24 June of 1976 through 1987.

Lag k	r_k	SE
1	−0.152	0.289
2	−0.012	0.295
3	−0.061	0.295
4	−0.245	0.296

Results

Stream Flows

An initial plot of $\log(R_{t+3}/S_t)$ versus D_t indicated that recruitment is related to the magnitude of stream flows (Fig. 4 and 5). Poor year-classes appear coincident to high flow events within the time-frame for flows. Conversely, strong year-classes appear coincident to years of low average stream flow during the time-frame (Fig. 4 and 5). Correlation analysis confirms the relation between $\log(R_{t+3}/S_t)$ and stream flow ($r = -0.751, P = 0.005$) during the 16 May through 24 June time-frame. Linear regression of $\log(R_{t+3}/S_t)$ on D_t was also significant (Table 3). Analysis of residuals indicated no heteroscedasticity, autocorrelation among residuals (Table 4), or undue influence of one or more residuals (Fig. 6). When other time-frames for stream flow were used, correlation and regression statistics remained significant and tended to increase as the time-frame for flows was increased beyond the hypothesized 24 June ending date (Table 3). The analysis for stream flow from 16 May through 10 June was deemed invalid, since parent stock was weakly correlated with average stream flow ($r = 0.649, P = 0.022$).

Stock Size

During 1979 to 1988, abundance of age 3 and older Arctic grayling had averaged 40 152 fish, while catch during this period had averaged 11 595 fish. Rates of instantaneous total mortality ranged from 0.34 in 1988 to 1.13 in 1985, averaging 0.76 from 1979 to 1988. Using an average rate of 0.76 for total mortality, the instantaneous rate of fishing mortality was 0.45. By subtraction, the instantaneous natural mortality rate was 0.31. When the discounted recruitment was summed across years, the observed stock size was exceeded (predicted S = 35 570 fish versus an observed S of 33 145 fish). Therefore, parent stock in 1976 was plausibly lower than the level of parent stock that might cause density dependence to occur. Furthermore, this result indicates that the level of parent stock that might cause density dependence is greater than 35 570 fish because some influence of stream flows is present in the observed recruitment.

Discussion

Although stream flows during the early life history of Arctic grayling do influence recruitment, the exact mechanisms that produce this influence are largely unknown. One consequence of high stream flow after spawning is disruption of the stream bottom, possibly dislodging eggs from the shallow nest that is typical of Arctic grayling (Vincent 1962; Elwood and Waters 1969). After hatching and emergence, the larvae of Arctic grayling have poorly developed fins and prefer low current velocities (Nelson 1954; Lee 1985). High stream flows during the larval stage could flush larvae downstream into areas of low food abundance (Crecco and Savoy 1987) or trap larvae in high water pools (Sytina 1964; Lee 1985). Water temperature is depressed by high stream flows, possibly affecting growth and survival of larvae through delayed hatching or decreased metabolic processes (Walker 1983). Turbidity increases with increasing stream flow in the Chena River (McFadden and Stallion 1979), lowering the feeding effectiveness and growth rate of young Arctic grayling (Walker 1983). Stream flow and its correlates may all influence survival rate of Arctic grayling during the period from egg deposition to the end of the larval stage.

Although correlation coefficients and regression statistics remained significant ($\alpha = 0.01$) as the time-frame for stream flows was extended, this result may be due to factors other than the length of the time-frame. Field validation of spawning, emergence, and duration of the larval stage would allow fine tuning of the optimum time-frame for calculating average stream flows. However, fine tuning of the time-frame would not necessarily imply that recruitment could be predicted from stream flows alone. Variations in mortality from egg to recruitment age could vary as a result of other factors, and the relation present in this study accounts for only 52–62% of the variability in recruitment. As Walters and Collie (1988) have succinctly illustrated, surveys designed to specifically measure year-class strength (e.g. catch rates of young-of-the-year) can yield better predictions of recruitment than regression models using an environmental influence discovered through correlation analysis.

As with any study involving estimated quantities, errors in estimation can confound efforts to differentiate between informative variation and measurement error. One know bias was that estimates of abundance and age composition were not adjusted for selectivity from 1976 through 1984. Electrofishing notoriously selects for the largest sizes of fish (Reynolds 1983). Therefore, these unadjusted estimates would likely bias recruitment downward while biasing parent stock upward. Clark (1991) found that mark–recapture techniques could be used to alleviate bias due to electrofishing, and that gone uncorrected, bias would have caused estimates of recruitment for the 1984 through 1987 year-classes to range from 0 to 25% low. Bias would have caused parent stock to range from 0 to 22% high. In this study, bias due to gear selectivity may have resulted in underestimates of $\log(R_{t+3}/S_t)$ for the 1976 through 1983 year-classes. Additionally, had these estimates been adjusted for selectivity, they most likely would have had estimates of measurement error calculated. Estimates of measurement error would have enabled a weighted regression of $\log(R_{t+3}/S_t)$ on D_t. Weighted regression, if possible, would have resulted in the same parameter estimates as unweighted least squares, but with an increase in the coefficient of determination (Draper and Smith 1981). Lack of a weighted regression to correct for heteroscedasticity in the data from the mark–recapture experiments implies that the influence of stream flows on recruitment is actually greater than indicated in this study.

Given the constraints of my analysis, there was no detectable influence of stock size on recruitment of Arctic grayling. Unfortunately, detection was restricted to a single year-class produced when the influence from stream flows was minimal. For a variety of reasons, I could not use other data pairs from year-classes produced during low stream flows to locate other points on the stock–recruitment relation. First, I have no sup-

portable hypothesis concerning the theoretical shape this relation might take (Ricker, Beverton–Holt, or some other stock–recruitment relation). Secondly, I have few estimates of measurement error for the stock and recruitment data pairs. Lastly, I have few data points at low stream flow conditions. Therefore, it would be very dangerous to attempt definition of the stock–recruitment relation without prior knowledge of the shape of the relation (Walters and Collie 1988) and without knowlege of measurement errors (Walters and Ludwig 1981).

However, the analysis of stock size provided useful information about the stock–recruitment relation of Arctic grayling in the Chena River. If, at high levels of parent stock, subsequent recruitment can theoretically replace and exceed that parent stock, then the level of parent stock that cannot replace itself must be logically higher. The level of parent stock that fails to replace itself cannot be estimated from available data, but some insight can be made by elevating levels of parent stock above the observed level of 33 415 fish.

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Crustacean Plankton in Lake Winnipeg: Variation in Space and Time as a Function of Lake Morphology, Geology, and Climate

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Rivers draining different geological basins have the most important impact on the formation of the planktonic community in Lake Winnipeg. Very diverse patterns of distribution of individual species reflected the complexity of the water masses structured by lake morphology and the configuration of river inflows. Of the 34 species identified (15 copepods and 19 cladocerans), 12 were found exclusively in the South Basin, 7 exclusively in the North Basin, and 15 were common to both basins. A "core" group of 12 species was distributed over the whole lake, but the remaining 22 species ("unsuccessful invaders") were present only in restricted areas, mostly adjacent to river inflows. Plankton species composition has not exhibited major changes after 40 yr, but abundance has at least doubled, probably due to eutrophication. Several times more planktonic crustaceans were found in the western part of the lake, affected by sedimentary drainage, than in the eastern part, influenced by the Precambrian Shield. The plankton community in a lake as large as Lake Winnipeg can be affected by differences in climate within its shores. Midsummer epilimnion temperature was the single best parameter predicting crustacean abundance in Lake Winnipeg and other North American great lakes and, combined with phosphorus loading, explained 97% of variance.

Les tributaires traversant différents bassins géologiques avant de se déverser dans le lac Winnipeg ont le plus important impact sur la communauté planctonique lacustre. Les régimes de répartition très divers des espèces traduisent la complexité des masses d'eau dont la structure est déterminée par la morphologie du lac et la configuration des influx fluviaux. Des 34 espèces de plancton identifiées (soit 15 espèces de copépodes et 19 espèces de cladocères), 12 espèces n'étaient présentes que dans le bassin méridional, 7 dans le bassin septentrional et 15 étaient communes à ces deux bassins. Un "noyau" de 12 espèces était réparti sur toute la superficie du lac, mais les autres 22 espèces ("des envahisseurs sans succès") n'étaient présentes que dans certains secteurs restreints, en particulier aux environs des influx fluviaux. La variété des espèces planctoniques n'a pas grandement changé au cours des 40 dernières années, mais leur abondance a au moins doublé, probablement en réaction à l'eutrophisation. Ainsi, l'abondance de crustacés planctoniques était plusieurs fois plus élevée dans le secteur occidental du lac qui reçoit des sédiments que le secteur oriental soumis à l'influence du Bouclier canadien. La communauté planctonique d'un lac aussi grand que le lac Winnipeg peut être touchée par des variations du climat local. La température de l'épilimnion au milieu de l'été s'est révélée le meilleur paramètre pour prédire l'abondance des crustacés dans le lac Winnipeg et d'autres grands lacs de l'Amérique du Nord et, ajoutée à la charge en phosphore, expliquait 97 % de sa variance.

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Great lakes are usually complex systems supplied with waters from different geologic areas and even from different climatic regions. It is logical to expect this complexity to influence abundance, distribution, and, perhaps, species composition of their planktonic communities. Small lakes tend to receive water from geologically and climatologically uniform drainage areas.

Lake Winnipeg is an example of a great lake, receiving water from sedimentary areas south and west of the lake via the Red and Saskatchewan rivers, respectively, and from the Precambrian Shield on the east drained mainly by the Winnipeg, Pigeon, Berens, and Poplar rivers. The lake extends between 50°0′ and 53°50′ north latitude and 96°15′ and 99°15′ west longitude, thus covering 3°30′ of latitude and 3°30′ of longitude. Within such a large area, significant climatic differences are observed and the responses of the planktonic community to these differences provide valuable information for predicting future responses of biotic systems to expected global climatic changes.

There is very little information available on the long-term changes of planktonic communities in such a large lake. The rare collections taken in the past have generally limited use for comparison with more recent samples. Bajkov's data from 1929 (Bajkov 1934) provided such a rare opportunity. The main objectives of this study were to (a) determine the species composition of the pelagic crustacean plankton, (b) examine the variation in space and time of zooplanktonic species, (c) compare the present data with historical records, and (d) understand how morphology, geology, and climate control the distribution and abundance of species within Lake Winnipeg.

Methods

A study of the pelagic crustacean plankton in Lake Winnipeg was carried out during June–October 1969 as part of a collaborative effort to characterize the chemical and biological conditions of the lake. A network of 51 stations was established to

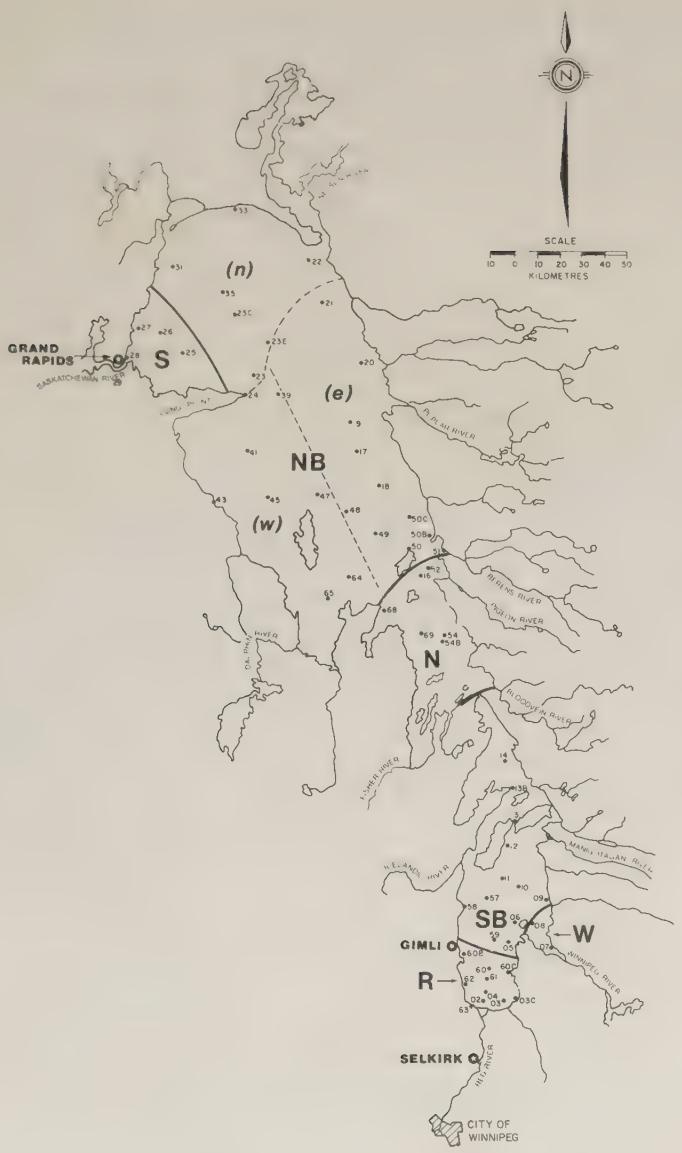

FIG. 1. Location of sampling stations and regionalization in Lake Winnipeg (R, Red River; W, Winnipeg River; SB, main part of the South Basin; N, Narrows; NB, North Basin with subdivisions e (east), w (west), and n (north); S, Saskatchewan River).

cover all areas of this large lake, 23 750 km^2 in surface area (Fig. 1). Zooplankton samples were obtained during six consecutive cruises, but due to weather conditions, sampling at all stations was not possible on all cruises: cruise 1, June 4–12, 36 stations; cruise 2, July 9–16, 51 stations; cruise 3, July 24 – August 1, 49 stations; cruise 4, September 2–10, 37 stations; cruise 5, October 3–13, 49 stations; cruise 6, October 27–31, 25 stations.

At each station, a Wisconsin net of 25-cm mouth diameter and 73-μm mesh size was hauled vertically from just above the lake bottom to the surface at a rate of approximately 0.5 m·s^{-1}. All the stations were sampled during the daytime hours. The plankton was preserved in 10% formalin. Measurement of settled net plankton volume was carried out prior to microscopic analysis. Graduated glass tubes, 8 × 600 mm, were used in which the plankton was allowed to settle for 24 h. At least 200 individuals per sample were counted and identified in a subsample containing about 1/40 of the whole sample. For larger and less numerous animals, either the whole or one fifth of the sample was examined. Identification of adult calanoids was made to species, while copepodids I–V were enumerated as calanoid juveniles and then apportioned amongst various species in accordance with the relative abundance of adults present in the sample. Copepodid stages of cyclopoids were identified to species. Nauplial stages of copepods were identified to family Cyclopidae and to suborder Calanoida. Zooplankton abundance was expressed as the number of specimens per litre, assuming that the filtration efficiency of the net was 100%. In fact, the efficiency was between 45 and 85%, averaging 63%, as indicated by later experiments.

Each basin was subdivided into regions (Fig. 1). Mean abundances in basins were calculated by weighting the zooplankton densities in each region according to the regional share of the total basin area. The morphology, chemistry, and temperature data were collected at the time by Brunskill (1974) and Brunskill et al. (1979a, 1980).

Lake Morphology, Hydrology, Geology, and Climate

Selected aspects of morphology, hydrology, geology, and climate of Lake Winnipeg and its drainage basins are summarized here to show the complexity of this aquatic system and its potential influence on the planktonic fauna inhabiting it.

The two parts of Lake Winnipeg, the South and North basins, are distinctly separated by the Narrows. The surface area of the North Basin is about six times greater than that of the South Basin, 17 520 and 2780 km^2, respectively. The Narrows cover an area of 3450 km^2. The mean depth of the North Basin is about one third greater than that of the South Basin, 13.3 and 9.7 m, respectively. The two basins differ greatly in the quantity and quality of water received.

There are three major sources of water supply to the lake. The Red River, with its main tributary the Assiniboine River, drains the areas south and west of the lake consisting of nutrient-rich sedimentary prairie with extensive agriculture and cities but few lakes. The Saskatchewan River, poorer in nutrients, drains the northern portion of the prairies with many lakes and reservoirs. The Precambrian Shield east of the lake, with its igneous bedrock overlain by variable thicknesses of Glacial Lake Agassiz derived soils, which support the growth of muskegs and boreal forests, is drained mainly by the Winnipeg, Manigotagan, Bloodvein, Pigeon, Berens, and Poplar rivers. This drainage area supports mining and forest industries, little agriculture, and has few large communities.

The South Basin receives 80% of its annual supply of water from the Precambrian Shield drainage of the Winnipeg River and only 20% from the sedimentary drainage of the Red River. However, this 20% supplies as much as 70% of the total phosphorus to the South Basin, since the concentration of phosphorus in the Red River is at least four times higher than in the Winnipeg River.

A reverse water supply pattern is observed in the North Basin, where 75% originates from the Saskatchewan River sedimentary drainage with only 25% from the Precambrian Shield. When inflow from the South Basin is added, the North Basin becomes a mixture of 60% from Precambrian Shield and 40% from the sedimentary drainage.

The richest source of nutrients to Lake Winnipeg is the Red River which supplies annually 60% of the phosphorus and 30% of the nitrogen. Another major source of sedimentary drainage water, the Saskatchewan River, is poor in nutrients, with total

TABLE 1. Seasonal changes of mean settled plankton volumes ($mm^3 \cdot L^{-1}$) in various regions of Lake Winnipeg, June–October 1969. The regions are designated in Fig. 1. Standard errors in parentheses.

	Region							
	South Basin				North Basin			
Date	R	W	SB	Mean	N	NB	S	Mean
June 4–12	6.35 (1.3)	2.77 (0.3)	7.13 (1.1)	6.83 (0.9)	4.57 (2.1)	7.16 (1.6)	5.14 (0.5)	6.86 (1.2)
July 9–16	9.20 (1.3)	2.30 (0.8)	12.83 (2.3)	11.44 (1.4)	2.64 (0.2)	5.41 (0.9)	8.38 (1.2)	5.42 (0.7)
July 24 – Aug. 1	12.38 (1.6)	2.77 (0.4)	8.32 (1.1)	8.44 (0.9)	5.48 (0.7)	4.71 (0.5)	6.85 (0.7)	4.20 (0.3)
Sept. 2–10	15.87 (3.2)	3.69 (0.0)	6.89 (0.9)	7.64 (1.2)	2.64 (0.2)	11.35 (1.6)	15.61 (2.5)	11.06 (1.4)
Oct. 3–13	6.98 (0.7)	0.92 (0.1)	3.33 (0.4)	3.58 (0.4)	2.44 (0.4)	9.95 (1.9)	8.38 (1.8)	9.39 (1.5)
Oct. 27–31	4.44 (1.3)	2.77 (0.3)	3.33 (0.5)	3.41 (0.4)	2.03 (0.2)	3.67 (0.7)	10.47 (3.2)	3.99 (0.8)

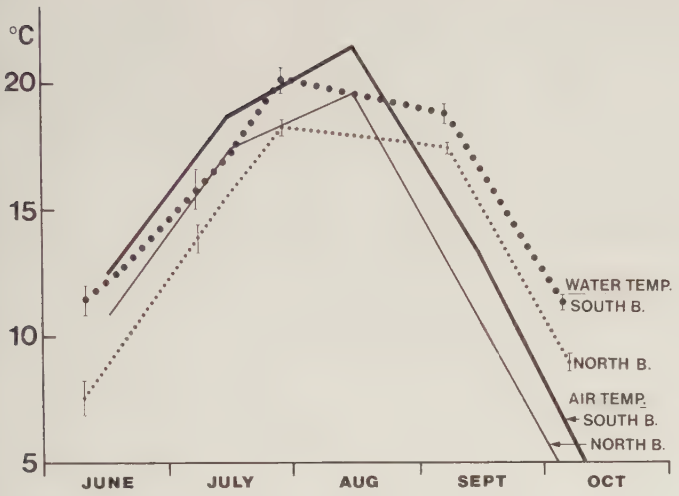

FIG. 2. Climatic differences between the northern and southern ends of Lake Winnipeg. The continuous lines denote the south and north end mean (± SE) monthly air temperatures; the dotted lines show the corresponding water temperatures (averaged over the upper 10-m layer).

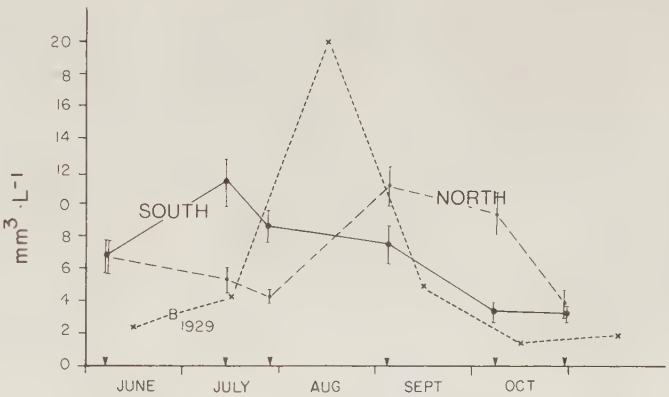

FIG. 3. Seasonal changes of mean (± SE) areal-weighted settled plankton volumes in the North and South basins of Lake Winnipeg. B denotes Bajkov's 1929 lake averages (Bajkov 1934).

phosphorus concentration one third that in the Red River. The above values are rough approximations of published records (Brunskill et al. 1979a).

Added to the complexities of water supply are the climatic differences between the northern and southern ends of the lake, which span 3.5° of latitude. Differences in air temperature between the north and south ends of the lake are observed in meteorological records from Grand Rapids and Gimli or Selkirk, respectively (Fig. 1 and 2) (Anonymous 1929–68). On average, air temperatures are 1.72°C warmer at the southern end of the lake, with north–south differences ranging from 1.0 to 2.6°C from June to November. Similarly, water temperatures of the upper 10-m stratum in the South Basin are on average 1.77°C warmer (range 1.2–4.2°C) than in the North Basin. Lake water temperatures lag behind air temperatures by about 6–12 d in both the June–August warming period and during the September–October cooling period. Another expression of climatic difference is the number of frost-free days, amounting to 100 in the North Basin and 120 in the South Basin (Anonymous 1929–68).

Results

Settled Net Plankton Volume

The simplest indication of planktonic biomass is the settled volume of material collected by the Wisconsin net in vertical hauls. Since the samples consist of both large phyto- and zooplankton in varying proportions, the method provides data for only a general characterization of lake productivity and spatial complexity (Table 1; Fig. 3). Although the seasonal average plankton volumes in the North and South basins were not much different (6.82 and 6.97 $mm^3 \cdot L^{-1}$, respectively), their seasonal distributions were. Maximum volumes of plankton in the North Basin were observed in September and October, i.e. 1–2 mo later than in the South Basin.

Plankton volumes varied considerably within each basin. Plankton was consistently poorest in area in Winnipeg River

TABLE 2. Seasonal changes in the abundance (ind.·L^{-1}) of planktonic crustaceans in the North and South basins of Lake Winnipeg, June–October 1969. Mean values are time-weighted. Blanks mean zero. See Salki and Patalas (1992) for details of individual stations.

Species	June 4–12		July 9–16		July 24 – Aug. 1		Sept. 2–10		Oct. 3–13		Oct. 27–31		Seasonal	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
							South Basin							
Limnocalanus macrurus Sars	0.030	0.010	0.003	0.001	0.001	0.004	0.003	0.001		0.001	0.001	0.001	0.005	0.002
Epischura lacustris Forbes	0.410	0.196	1.100	0.243	2.500	0.582	0.790	0.181	0.150	0.035	0.010	0.003	0.948	0.144
Epischura nevadensis Lilljeb.	1.220	0.459	0.830	0.152	4.800	0.709	0.390	0.081	0.010	0.005	0.010	0.001	1.313	0.199
Diaptomus ashlandi Marsh	4.550	1.099	9.380	1.430	22.590	5.539	26.280	6.710	6.310	1.082	3.010	1.247	14.471	1.861
Diaptomus oregonensis Lilljeb.	0.100	0.229	1.210	0.263	1.150	0.643	1.930	0.588	2.540	0.516	0.220	0.088	1.420	0.213
Diaptomus siciloides Lilljeb.	0.210	0.088	0.020	0.029	0.020	0.073	2.150	5.949	0.350	0.504	0.140	0.400	0.643	1.080
Diaptomus sicilis Forbes														0.005
Diaptomus minutus Lilljeb.														
Diaptomus leptopus Forbes									0.001	0.003	0.001	0.001		0.001
Diaptomus clavipes Schacht			0.001											
Diaptomidae nauplii	6.180	1.184	26.420	3.104	33.130	6.844	13.400	4.210	3.380	0.524	0.400	0.288	15.765	2.022
Diacyclops bicuspidatus thomasi Forbes	6.790	0.939	2.040	0.392	1.870	0.319	0.140	0.090	0.110	0.048	0.480	0.346	1.630	0.230
Acanthocyclops vernalis Fischer	6.150	2.418	3.480	0.953	3.420	3.462	7.370	3.612	2.370	0.720	0.970	0.390	4.360	1.009
Mesocyclops edax (Forbes)							0.001	0.001	0.001					
Macrocyclops albidus (Jurine)									0.001					
Eucyclops agilis (Koch)	0.040	0.022	0.200	0.132			0.001	0.001					0.041	0.029
Cyclopidae nauplii	17.740	4.035	7.110	2.018	12.610	7.701	8.730	4.638	3.960	0.789	1.350	0.473	8.826	1.841
Daphnia retrocurva Forbes	0.150	0.066	7.340	1.257	4.320	2.473	0.740	0.354	0.100	0.075	0.010	0.003	2.350	0.606
Daphnia galeata mendotae Birge	0.010	0.002	0.010	0.035	0.140	0.236	3.810	1.222	0.330	0.140	0.050	0.081	1.041	0.273
Daphnia longiremis Sars														
Daphnia parvula Fordyce	0.010	0.001					0.010	0.008	0.020	0.012			0.007	0.003
Daphnia ambigua Scourfield		0.000	0.010	0.016									0.002	0.003
Daphnia pulex Leydig	0.001	0.002	0.001	0.001	0.100	0.056							0.019	0.010
Daphnia schoedleri Sars	0.010	0.004	0.010	0.034									0.003	0.007
Ceriodaphnia quadrangula (Mull.)	0.060	0.016	0.010	0.023			0.060	0.262	0.010	0.014			0.026	0.047
Simocephalus vetulus Schoedler														
Bosmina longirostris (Muller)	0.570	0.127	4.430	0.831	1.910	0.785	0.390	0.092	1.550	0.221	0.520	0.301	1.647	0.263
Chydorus sphaericus (Muller)														
Holopedium gibberum Zaddach							0.001	0.001						
Diaphanosoma leuchtenbergianum Fischer	0.020	0.007	0.180	0.083	1.560	0.785	1.170	0.719	0.150	0.025	0.010	0.005	0.652	0.216
Sida crystallina (Muller)									0.001					
Leptodora kindtii (Focke)	0.003	0.002	0.050	0.010	0.100	0.055	0.010	0.010	0.001	0.001			0.031	0.011
Alona guttata Sars		0.000	0.040	0.019									0.007	0.004
Leydigia quadrangularis (Leydig)					0.150	0.112	0.001	0.001	0.010	0.011	0.001	0.001	0.031	0.021
Eurycercus lamellatus (Muller)														
Latona setifera (Muller)				0.001										
Total	44.250	7.381	63.880	5.155	90.370	21.535	67.380	24.664	21.360	3.290	7.180	3.147	55.241	6.938
Number of species	18		20		15		19		19		14		27	

TABLE 2. (*Concluded*)

Species	June 4–12		July 9–16		July 24 – Aug. 1		Sept. 2–10		Oct. 3–13		Oct. 27–31		Seasonal	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
							North Basin							
Limnocalanus macrurus Sars	0.270	0.069	0.110	0.025	0.090	0.029	0.040	0.011	0.030	0.009	0.040	0.009	0.088	0.014
Epischura lacustris Forbes	0.040	0.031	0.310	0.046	0.350	0.143	0.320	0.072	0.050	0.023	0.010	0.004	0.216	0.048
Epischura nevadensis Lilljeb.	0.160	0.006	0.310	0.164	0 350	0.157	0.130	0.042	0.010	0.003	0.010	0.003	0.176	0.053
Diaptomus ashlandi Marsh	1.910	0.380	3.610	0.951	5.400	1.049	7.760	0.985	4.480	0.608	5.930	1.639	5.114	0.414
Diaptomus oregonensis Lilljeb.			0.050	0.042	0.170	0.122	1.030	0.239	0.760	0.168	0.110	0.054	0.448	0.062
Diaptomus siciloides Lilljeb.			0.010	0.004	0.020	0.022	0.160	0.105	0.040	0.024			0.053	0.016
Diaptomus sicilis Forbes				0.024	0.040	0.010	0.100	0.040	0.050	0.017	0.140	0.009	0.052	0.008
Diaptomus minutus Lilljeb.				0.004	0.100	0.075	0.420	0.159	0.030	0.013			0.129	0.029
Diaptomus leptopus Forbes														
Diaptomus clavipes Schacht														
Diaptomidae nauplii	4.590	0.635	7.290	1.830	11.940	1.497	11.620	1.353	1.740	0.232	0.640	0.184	7.391	0.645
Diacyclops bicuspidatus thomasi Forbes	2.620	0.493	16.350	4.461	17.300	3.256	10.200	1.508	2.200	0.454	1.100	0.213	9.555	1.294
Acanthocyclops vernalis Fischer	0.670	0.158	0.640	0.212	1.670	0.280	6.300	0.989	6.570	0.917	3.090	0.752	3.543	0.313
Mesocyclops edax (Forbes)							0.010	0.021					0.002	0.003
Macrocyclops albidus (Jurine)														
Eucyclops agilis (Koch)														
Cyclopidae nauplii	12.050	3.607	25.350	6.593	17.450	2.231	28.810	3.892	8.960	1.411	6.910	2.303	18.639	1.999
Daphnia retrocurva Forbes	0.020	0.001	0.350	0.100	1.560	0.255	0.730	0.213	0.030	0.010	0.010	0.002	0.551	0.084
Daphnia galeata mendotae Birge	0.020	0.003	0.140	0.078	0.820	0.224	1.400	0.272	0.780	0.221	0.080	0.027	0.684	0.085
Daphnia longiremis Sars	0.010	0.009	0.200	0.193	0.680	0.292	0.450	0.231	0.040	0.021		0.001	0.286	0.085
Daphnia parvula Fordyce														
Daphnia ambigua Scourfield														
Daphnia pulex Leydig														
Daphnia schoedleri Sars														
Ceriodaphnia quadrangula (Mull.)							0.010	0.001					0.002	
Simocephalus vetulus Schoedler							0.001	0.010						0.001
Bosmina longirostris (Muller)	0.280	0.125	3.640	0.878	4.440	0.854	0.320	0.534	1.280	0.668	0.370	0.271	1.877	0.324
Chydorus sphaericus (Muller)	0.050	0.093	0.030	0.027	0.160	0.162	0.910	0.850	2.390	0.855	0.080	0.045	0.723	0.210
Holopedium gibberum Zaddach														
Diaphanosoma leuchtenbergianum Fischer	0.010		0.020	0.034	0.170	0.101	0.700	0.128	0.160	0.043	0.010	0.003	0.242	0.034
Sida crystallina (Muller)														
Leptodora kindtii (Focke)	0.003	0.001	0.007	0.004	0.050	0.012	0.010	0.004	0.003	0.002			0.014	0.003
Alona guttata Sars														
Leydigia quadrangularis (Leydig)														
Eurycercus lamellatus (Muller)									0.001					
Latona setifera (Muller)			0.001											
Total	22.700	4.230	58.420	10.953	62.780	5.855	71.400	7.832	29.560	3.661	18.530	4.994	49.776	3.356
Number of species	13		16		17		20		18		13		22	

TABLE 3. Mean abundance (ind.·L^{-1}) of species found in the North and/or South basins of Lake Winnipeg, June–October 1969. Zeros in the North and South columns indicate that the species were not found. Standard errors in parentheses.

	North (N)	South (S)	Ratio N/S
Chydorus sphaericus	0.72 (0.22)	0	—
Daphnia longiremis	0.29 (0.08)	0	—
Diaptomus minutus	0.13 (0.04)	0	—
Diaptomus sicilis	0.05 (0.02)	0	—
Eurycercus lamellatus	0.001	0	—
Latona setifera	0.001	0	—
Simocephalus vetulus	0.001	0	—
Limnocalanus macrurus	0.09 (0.01)	0.005 (0.002)	16.7
Diacyclops bicuspidatus thom.	9.6 (1.63)	1.6 (0.23)	6.0
Bosmina longirostris	1.88 (0.27)	1.65 (0.26)	1.1
Mesocyclops edax	0.002	0.001	1.0
Acanthocyclops vernalis	3.54 (0.39)	4.36 (1.00)	0.8
Daphnia galeata mendotae	0.68 (0.09)	1.04 (0.27)	0.7
Diaptomus ashlandi	5.11 (0.42)	14.47 (1.86)	0.4
Diaphanosoma leuchtenbergianum	0.24 (0.05)	0.65 (0.22)	0.4
Leptodora kindtii	0.01 (0.003)	0.03 (0.011)	0.33
Diaptomus oregonensis	0.45 (0.07)	1.42 (0.21)	0.32
Epischura lacustris	0.22 (0.05)	0.95 (0.14)	0.23
Daphnia retrocurva	0.55 (0.08)	2.35 (0.61)	0.23
Epischura nevadensis	0.18 (0.05)	1.31 (0.20)	0.14
Ceriodaphnia quadrangula	0.002	0.02	0.10
Diaptomus siciloides	0.05 (0.02)	0.64 (0.60)	0.08
Eucyclops agilis	0	0.04 (0.028)	0
Leydigia quadrangularis	0	0.03 (0.020)	0
Daphnia pulex	0	0.01 (0.010)	0
Alona gutata	0	0.01	0
Daphnia parvula	0	0.007	0
Daphnia schoedleri	0	0.003	0
Holopedium gibberum	0	0.001	0
Daphnia ambigua	0	0.002	0
Macrocyclops albidus	0	0.001	0
Diaptomus clavipes	0	0.001	0
Diaptomus leptopus	0	0.001	0
Sida crystallina	0	0.001	0

area and richest near the Red River inflow, with mean volumes almost four times higher than the Winnipeg River area. The richest area of the North Basin was adjacent to the Saskatchewan River inflow.

Species Composition

A total of 34 crustacean species (19 cladocerans and 15 copepods) were identified in Lake Winnipeg samples from June to October 1969 (Table 2). The number of species in the planktonic community remained relatively constant in both basins of Lake Winnipeg throughout the sampling season. During individual cruises, from 14 to 20 species of crustaceans were present in the South Basin and from 13 to 20 species in the North Basin. Twelve species were found exclusively in the South Basin (Table 3). All of these species were found mainly in the nearshore area close to the Red River delta. Seven species were found exclusively in the North Basin (Table 3), most of them in the vicinity of the Saskatchewan River inlet. Most species exclusive to one basin were found only in very small numbers, rarely exceeding 0.1% of the total number of crustaceans in that basin. Exceptions were *Daphnia longiremis* and *Chydorus sphaericus* in the North Basin, amounting to 0.5 and 1.4%, respectively.

Three copepods *Diacyclops bicuspidatus thomasi*, *Diaptomus ashlandi*, and *Acanthocyclops vernalis* were the most abundant species found during the June–October 1969 study period, representing 46.1, 24.2, and 17.6%, respectively, of the total crustaceans in the North Basin and 10.5, 48.7, and 17.9% in the South Basin.

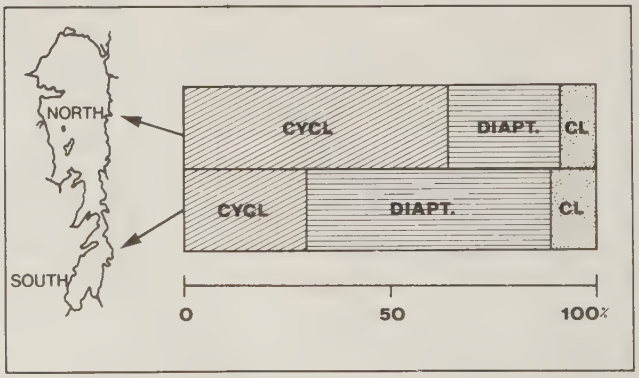

FIG. 4. Mean percentage of cyclopoid, diaptomid, and cladoceran abundance in the crustacean community in the North and South basins of Lake Winnipeg.

Cladocerans were not nearly as numerous. The four most abundant species were *Daphnia retrocurva* representing 1.0 and

4.3% of the total abundance in North and South basins, respectively, *Bosmina longirostris* with 3.9 and 3.2%, *Daphnia galeata mendotae* with 1.2 and 1.5%, and *Diaphanosoma leuchtenbergianum* with 0.4 and 1.1%.

The generalized structure of the major components of the Lake Winnipeg planktonic communities (Fig. 4) indicates that filtrators (diaptomids and cladocerans) were numerically predominant in the South Basin (71.5%), while omnivores and predators (cyclopoids) dominated the North Basin (63.8%).

Seasonal Changes in the Horizontal Distribution and Abundance of Planktonic Crustaceans

In spite of the intensive vertical water column mixing in Lake Winnipeg, the horizontal distribution of water masses and planktonic crustaceans was far from uniform. On many occasions, large differences in zooplankton densities existed among stations. These significant differences persisted throughout the season for some species, while densities varied from month to month for others. This complexity of the spatial distribution of individual species complicates the task of analysis of seasonal changes in abundance of these species. Sampling at the same stations over time does not necessarily provide information on the seasonal history of a population because the water mass in which a population lives may be affected by variable river discharges and wind speed and direction which cause it to move in unpredictable ways. Water mass boundaries and their consecutive positions cannot be easily established. Therefore, one must average over larger areas such as the South and North basins to provide reasonable values reflecting seasonal changes in plankton abundance. As the average net water residence times in the South and North basins (0.5 and 2.5 yr, respectively; Brunskill et al. 1980) were very much longer than the sampling intervals, one could assume that the same water mass was often sampled regardless of its actual location within the basin.

In the South Basin, total numbers of crustaceans during the first half of the open-water season were from 10 to 100% greater than in the North Basin (Fig. 5). Initial (June) densities were about twice as high in the South Basin as in the North Basin (44 and 23 ind.·L^{-1}, respectively). From June to August, numbers steadily increased at about the same rate in both basins. The South Basin peak preceded that in the North Basin by about 1 mo. From September to October, crustacean abundance decreased in both basins. At the end of the season in October, numbers in the South Basin were about half those of the North Basin, a reversal of the pattern during the first half of the open-water season. The patterns of seasonal changes of planktonic crustaceans were mainly produced by three copepod species. *D. ashlandi, D. bicuspidatus thomasi*, and *A. vernalis* contributed more than 80% to the total number of crustaceans, while all 19 species of cladocerans constituted no more than 5% of the total. The three dominant copepods, however, exhibited marked differences in their developmental patterns in the two basins.

Copepods

Diaptomus ashlandi (Fig. 6a) was most abundant in the South Basin, particularly in the vicinity of the Red River inlet (stations 2 and 3) and along the western shore (station 58), reaching levels of 98.8 ind.·L^{-1} in July. It was relatively scarce in the Winnipeg River – Traverse Bay area (stations 7 and 8), never exceeding 1.6 ind.·L^{-1}. North Basin abundances were usually two to five times lower than those of the South Basin, with a tendency to increase southward. Frequently, the highest numbers within the North Basin were observed along the eastern shore, particularly in the vicinity of stations 20 and 21 (23.7 ind.·L^{-1} in July and 25.8 ind.·L^{-1} in October).

Diaptomus ashlandi reached maximum abundance in the South Basin by late July but declined to a minimum by October (Fig. 5). In the North Basin, the rate of increase was much slower, with a slight peak in September and a slight increase in October.

Diaptomus oregonensis (Fig. 6b) appeared in June near the Red River mouth and spread gradually throughout the South Basin except near Traverse Bay, where it was always absent. In the North Basin, this species appeared initially in July in the Pigeon Bay area (stations 16 and 52) and at the Saskatchewan River mouth (station 28), spreading by midsummer over the entire basin but existing in very low numbers. *Diaptomus oregonensis* barely reached 1/10 the density of *D. ashlandi*. More abundant in the South Basin, it gradually increased in numbers, reaching a peak in September and early October. In the North Basin, its development was marked by the characteristic delay, being absent there in June and July (Fig. 5).

Diaptomus siciloides (Fig. 6c) was found in rather restricted areas, occurring near the mouths of the Red and Saskatchewan rivers in abundance generally below 5 ind.·L^{-1}. Only at station 2 near the Red River in September did it reach an abundance as high as 11.7 ind.·L^{-1}. In late July and October, it was also found in the area adjacent to the Warpath River south of Long Point. It was about 10 times more numerous in the South Basin than in the North Basin (Fig. 5).

Diaptomus sicilis (Fig. 7a) occurred in very low numbers throughout the season, being found mainly in the autumn along the eastern shore of the North Basin (stations 20 and 21) and in an area around Berens Island (station 50). It was absent in the South Basin.

Diaptomus minutus (Fig. 7b) was restricted to the northern part of the North Basin associated mostly with the Saskatchewan River mouth area.

Diaptomus leptopus was found only in October and only in a few stations located near the Red River inflow.

Diaptomus clavipes was present as a single specimen recorded in early July near the mouth of the Red River.

Epischura lacustris (Fig. 7c) and *Epischura nevadensis* (Fig. 8a) were found all over the lake but were, on average, about four to eight times more abundant in the South Basin than in the North Basin. Their distribution was confined mainly to the central and southern parts of the South Basin where abundances were at least 10–20 times higher than those in the North Basin. While in most cases both species were found together in the same sample, *E. nevadensis* was almost twice as abundant as *E. lacustris* in the South Basin. In the North Basin, the numbers of both species were similar. The copepodids of both species (Fig. 8b) were, as their adult forms, more abundant in the South Basin, but as a rule, they were absent in the plumes of the Winnipeg and Red rivers. *Epischura lacustris* and *E. nevadensis* displayed similar seasonal patterns. Their numbers, low at the beginning of the open-water season, reached a maximum in July and declined thereafter.

Diaptomus and *Epischura nauplii* (Fig. 8c) were distributed all over the lake throughout the summer, but maximal numbers occurred in the South Basin, particularly in the area adjacent to the Red River, with 135.5 ind.·L^{-1} in July, densities about 10 times higher than those in the North Basin. They showed a

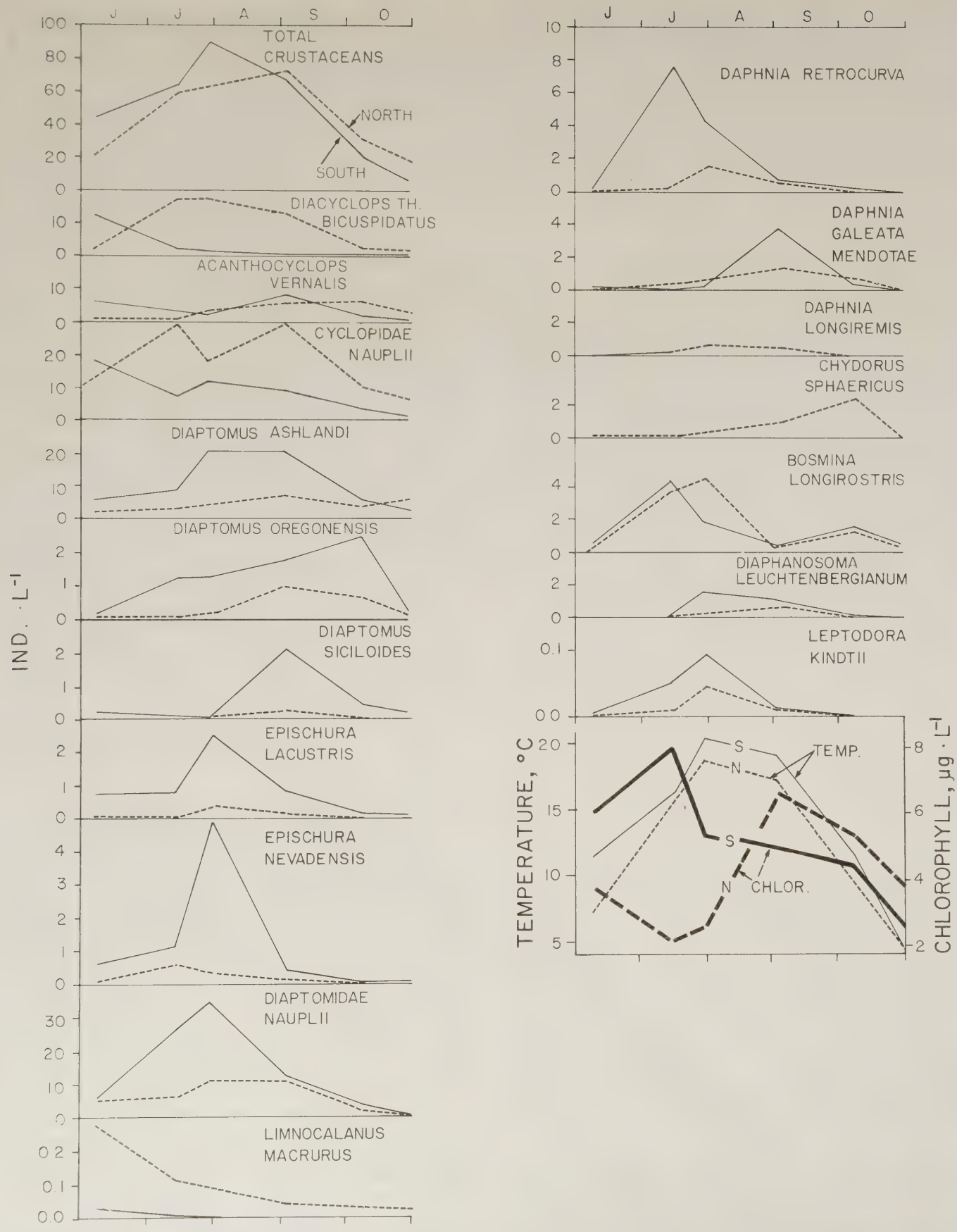

FIG. 5. Changes in the abundance of various species of crustaceans, mean temperature at 0–10 m, and chlorophyll *a* concentration in the North (N) and South (S) basins, June–October 1969.

FIG. 6. Spatial distribution (ind.·L^{-1}) of adults and copepodids of (a) *Diaptomus ashlandi*, (b) *Diaptomus oregonensis*, and (c) *Diaptomus siciloides* in Lake Winnipeg, June–October 1969.

FIG. 7. Spatial distribution (ind.·L^{-1}) of adults and copepodids of (a) *Diaptomus sicilis*, (b) *Diaptomus minutus* and (c) adults of *Epischura lacustris* in Lake Winnipeg, June–October 1969.

FIG. 8. Spatial distribution (ind.·L^{-1}) of (a) adults of *Epischura lacustris*, (b) copepodids of *Epischura* sp., and (c) nauplii of *Diaptomus* sp. and *Epischura* sp. in Lake Winnipeg, June–October 1969.

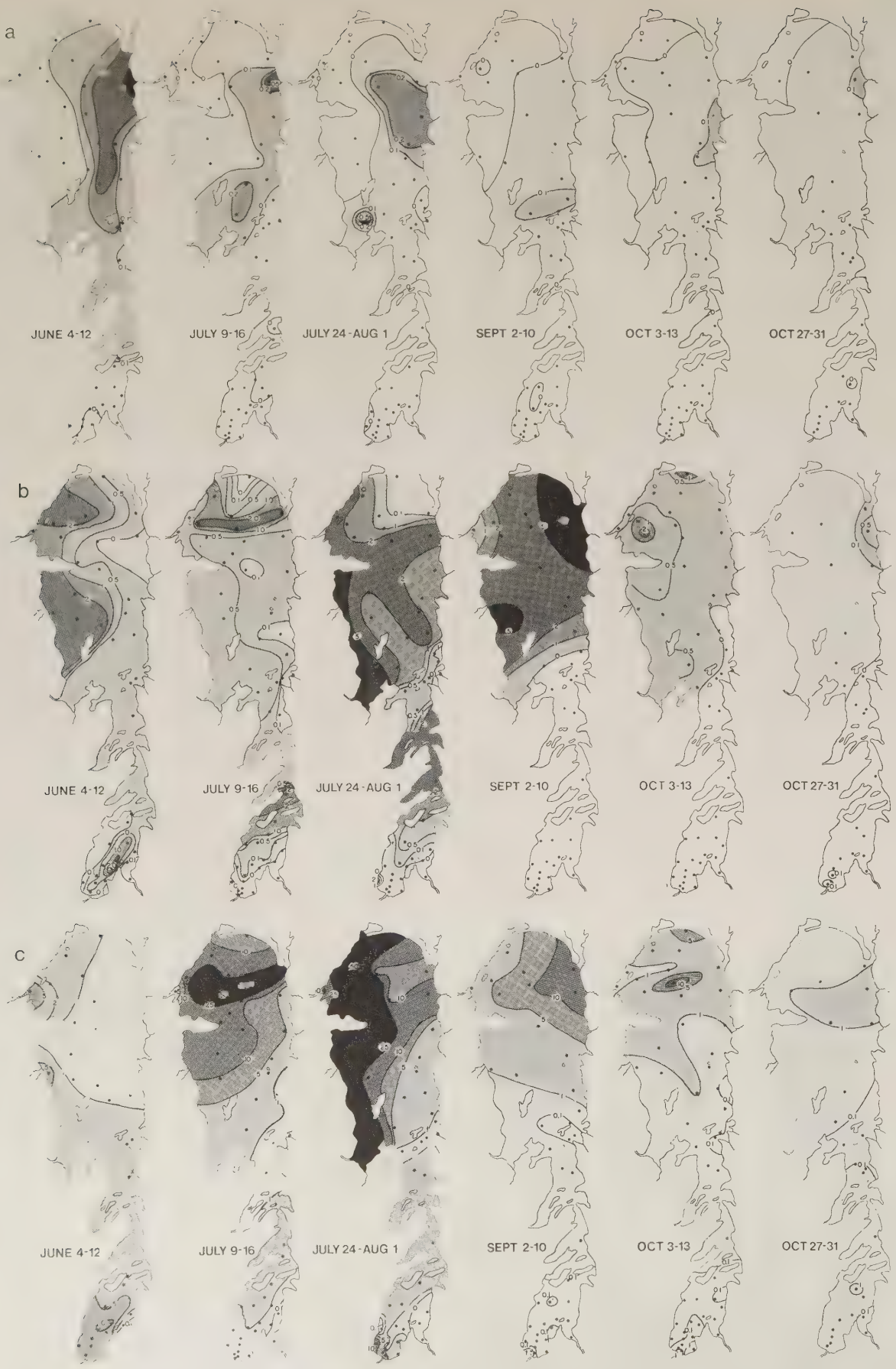

FIG. 9. Spatial distribution (ind.·L^{-1}) of (a) adults and copepodids of ***Limnocalanus macrurus***, (b) adults of *Diacyclops bicuspidatus thomasi*, and (c) copepodids mainly of ***Diacyclops bicuspidatus thomasi*** in Lake Winnipeg, June–October 1969.

seasonal distribution very closely resembling that of *D. ashlandi*, with a July maximum in the South Basin and a July–September maximum in the North Basin.

Limnocalanus macrurus (Fig. 9a) was present in June all over the lake with the exception of the area adjacent to the Red River inlet. The highest densities (1.0 ind.·L^{-1}) were found mainly in the deepest part of the North Basin, a strip running from Reindeer and Berens islands (stations 47 and 50) to the Nelson River outflow (station 22). The species gradually disappeared from the South Basin over the course of the summer but continued to maintain higher levels in the deepest, eastern portion of the North Basin. Very few specimens were encountered in the area adjacent to Saskatchewan River inlet. Mean abundance of *L. macrurus* in the North Basin continuously decreased throughout the summer (Fig. 5). Similar patterns were found in the South Basin, but with very low initial numbers, *L. macrurus* almost completely disappeared from this part of the lake by September. The nauplial stages were found only in June and only in the deepest part of the North Basin.

Diacyclops bicuspidatus thomasi (Fig. 9b and 9c) was the most abundant crustacean in the summer plankton of Lake Winnipeg. Three centres of abundance could be identified in June: one in the central part of the South Basin, the second in the North Basin between Reindeer Island and Long Point, and the third north of Long Point. In the South Basin (Fig. 5), highest densities occurred in the first month of sampling, June, suggesting that perhaps maximum population densities may have been reached prior to this date. Mean numbers in the South Basin gradually decreased during July, and by September, both adults and copepodids had almost completely disappeared (see also Fig. 9b and 9c). In contrast, the North Basin population was lower in abundance in June and reached a maximum in July, at least 1 mo later than in the South Basin. Numbers gradually decreased during September and October, but never to the low levels observed in the South Basin. On average, the North Basin population was four to five times more abundant than the South Basin population.

Acanthocyclops vernalis (Fig. 10a), second to *D. bicuspidatus thomasi* in abundance, demonstrated a very different pattern of distribution. Throughout June and July, highest numbers were always found in the most southern portion of the South Basin, in the immediate vicinity of the Red River inflow. At the same time, areas adjacent to the Winnipeg River were almost completely devoid of *A. vernalis* as well as all other crustaceans. In the North Basin, *A. vernalis* was found everywhere during the summer, but usually in quantities lower than those in the South Basin (Fig. 5). By October, the pattern was reversed, with about three times more animals found in the North Basin.

Mesocyclops edax was found only sporadically in September and October in the areas adjacent to the mouths of the Saskatchewan, Red, and Winnipeg rivers.

Eucyclops agilis was encountered in very small numbers in June and early July, exclusively in the South Basin, mainly in the areas adjacent to the Red and Winnipeg River mouths.

Macrocyclops albidus was found only once in October near the Winnipeg River mouth.

Cyclopidae nauplii (Fig. 10b and 5), common to all areas of the lake, had densities mostly between 10 and 50 ind.·L^{-1}. The exception was, however, the area adjacent to the Red River, with abundances much higher than lake average. In contrast, nauplii were almost entirely lacking in the Traverse Bay – Winnipeg River mouth area throughout the investigation. Cyclopidae nauplii demonstrated two pulses, in June and in late July in the South Basin and in July and September, after about 1-mo delay, in the North Basin (Fig. 5). On average, their densities were about twice as high in the North Basin than in the South Basin, as was overall cyclopoid abundance. In both basins, nauplii amounted to 59% of the total cyclopoid population.

Cladocerans

Daphnia retrocurva (Fig. 11a and 5) was the most abundance cladoceran species in Lake Winnipeg, although it contributed only 1 and 4% to the total number of crustaceans in the North and South basins, respectively. In comparison, the dominant copepod species, *D. ashlandi*, was about 10 times more abundant. *Daphnia retrocurva* appeared initially in June around the Red River inlet in the South Basin and at the few stations in the North Basin located near the Saskatchewan and Pigeon River mouths. By early July, the species had spread throughout the lake, reaching abundances in the South Basin which were 20 times higher than in the North Basin. Low abundances were always associated with the Nelson River outlet area in the eastern part of the lake. Numbers declined to very low levels by September, and by October, *D. retrocurva* disappeared from most regions of the lake except for small pockets near the Red and Saskatchewan River mouths.

Daphnia galeata mendotae (Fig. 11b and 5) was found in June in most parts of the lake in very low numbers, less than 0.1 ind.·L^{-1}. A gradual increase was evident throughout the lake in July, and by September, a maximum of 1 and 3 ind.·L^{-1} in the North and South basins, respectively, was reached. In some stations around the Red and Saskatchewan river inlets, densities of 5–15 ind.·L^{-1} were found. The population diminished in late October to very low numbers, usually less than 0.1 ind.·L^{-1}. The abundance of *D. galeata mendotae* was usually greater in the North Basin.

Daphnia longiremis (Fig. 11c and 5) was confined to the North Basin, at a centre of distribution associated with the Saskatchewan River inlet area where, in July, numbers reached a maximum of 1–6 ind.·L^{-1}. From this region, a gradual eastward spread occurred during June and July. In September and October, the species' centre of distribution moved along the northern shore towards the Nelson River outlet.

The following four daphnids were observed only as single specimens per sample: *Daphnia parvula* (Fig. 10c, A) in four stations located in close proximity to the Red River inlet from June to October, *Daphnia ambigua* (Fig. 10c, B) only once in July, close to the Red River inlet, and *Daphnia pulex* (Fig. 10c, C) and *Daphnia schoedleri* (Fig. 10c, D) in June and early July in 10 stations located in the central and southern parts of the South Basin. The taxonomic position of some individuals of the latter two species was not always clear. Many specimens with intermediate features between these two species were frequently found.

Ceriodaphnia quadrangula (Fig. 12a) was restricted to the South Basin, most frequently found around the Red River inlet where, in September, the highest density of 5 ind.·L^{-1} was encountered.

Simocephalus vetulus was found once as a single specimen in September near the Saskatchewan River inlet.

Bosmina longirostris (Fig. 12b) was distributed throughout the lake for most of the season, with abundances ranging

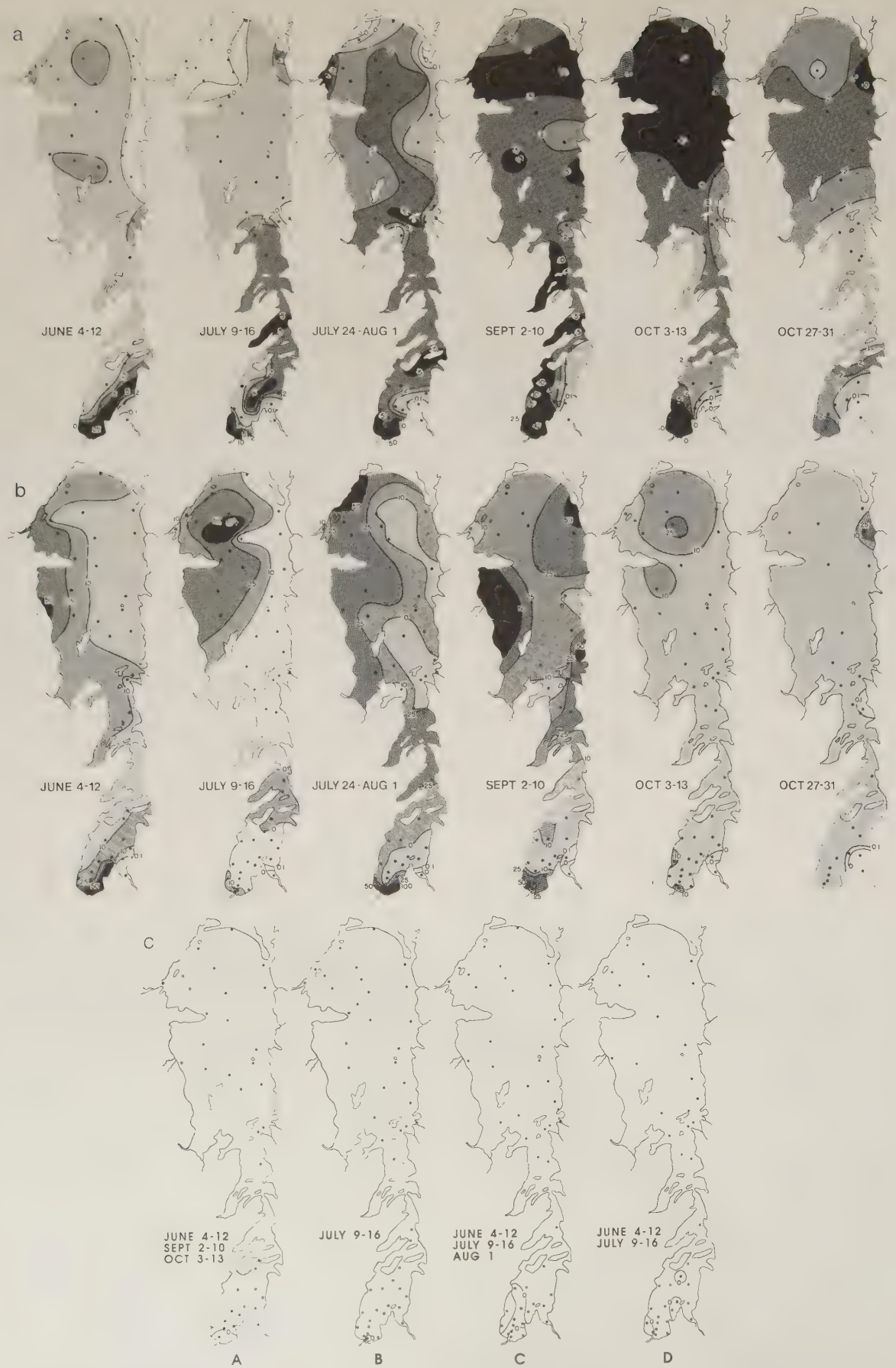

FIG. 10. Spatial distribution (ind.·L^{-1}) of (a) adults and copepodids of *Acanthocyclops vernalis*, (b) nauplii of Cyclopidae, and (c) single specimens of *Daphnia parvula* (A), *Daphnia ambigua* (B), *Daphnia pulex* (C), and *Daphnia schoedleri* (D) in Lake Winnipeg, June–October 1969.

FIG. 11. Spatial distribution of (ind.·L^{-1}) of adults and juveniles of (a) *Daphnia retrocurva*, (b) *Daphnia galeata mendotae*, and (c) *Daphnia longiremis* in Lake Winnipeg, June–October 1969.

between 1 and 5 ind.·L^{-1}. Two main centres of abundance with up to 17 ind.·L^{-1} were apparent, one in the vicinity of the Red River and the other near the Saskatchewan River inlet. Numbers were minimal in Traverse Bay and stations along the northeastern shores of the lake for most of the period. *Bosmina longirostris* (Fig. 5) reached its maximum abundance in early and late July in the South and North basins, respectively. Following a decline in September, a second smaller peak was observed in early October in both basins.

Chydorus sphaericus (Fig. 12c), like *D. longiremis*, was encountered only in the northern part of the North Basin, localized near the Saskatchewan River mouth and in the area between Reindeer Island and Long Point peninsula. Even during the period of its maximum abundance in September and October, this species did not expand beyond these regions. In late October, *C. sphaericus* occupied only a narrow strip extending between the Saskatchewan River inlet and the Nelson River outlet.

Leptodora kindtii (Fig. 13a) occurred in June in areas adjacent to the Saskatchewan, Red, and Pigeon River inflows. By late July it had spread over the entire lake, with maximum densities of 0.2–0.5 ind.·L^{-1} occurring in nearshore waters. Population counts declined in September, with a marked absence of specimens from central areas of both the North and South basins. In early October, only a few *Leptodora* were found near the Red and Saskatchewan River inflows, and by late October the species had completely disappeared from the lake water. *Leptodora* were never found in the Winnipeg River mouth area. South Basin numbers averaged three times higher than those in the North Basin (Fig. 5).

Diaphanosoma leuchtenbergianum (Fig. 13b) was found first in the South Basin and then spread throughout the lake in midsummer. Two areas of maximum densities were recorded, the first near the Red River inlet and the second in the North Basin in a strip between the Saskatchewan River inflow and Nelson River outflow. *Diaphanosoma leuchtenbergianum* was never found near the Winnipeg River inflow, and only a few specimens were encountered within the Pigeon River inflow. *Diaphanosoma leuchtenbergianum* appeared in Lake Winnipeg relatively later than most other crustacean species (Fig. 5). In the South Basin it reached maximum densities in late July, while in the North Basin it crested 1 mo later in September. On the average, three times greater densities were found in the South Basin than in the North Basin.

Leydigia quadrangularis was encountered from July to October only in a narrow strip adjacent to the Red and Winnipeg River inflows.

Sida crystallina and *Latona setifera* were found only once in October, and *Alona guttata* was found in June near the Red River inlet and *Eurycercus lamellatus* in October in the North Basin. *Holopedium gibberum* was found only once in September near the Winnipeg River inflow.

The spatial distribution of the entire crustacean community (Fig. 13c) exhibited a rather definite, nonrandom pattern. In the western part of the North Basin, particularly in the vicinity of the Saskatchewan river inflow, high numbers of crustaceans were found in spring and summer, on average 5–20 times higher than in the eastern part. A more uniform distribution throughout the entire basin was observed in September and October, when zooplankton abundance decreased gradually with decreasing water temperature.

In the South Basin, high plankton densities were observed in the central and southern parts, particularly in the vicinity of the Red River inflow. Conversely, the Winnipeg River – Traverse Bay area was always poor in crustaceans, with counts 100 times lower than those in the Red River inflow region. As a result of the Winnipeg River impoverishing effect, the northern part of the South Basin was always lower in numbers than the southern portion of the basin.

Discussion

Thirty-four species of planktonic crustaceans were found in Lake Winnipeg during the open-water period from June to October, and as many as 30 of these species occurred in the summer months (July to the beginning of September; Table 2). The number can be reduced to 27 by subtracting some littoral chydorids. This is a rather high number of species, since the comparable numbers for other North American great lakes are lower, i.e. Lake Superior, 18; Lake Huron, 23; Lake Ontario, 23; Lake Erie, 21–25 (Patalas 1972). The analysis of the individual maps of species distribution (Fig. 6a–13b) revealed, however, a planktonic community in Lake Winnipeg with a very specific spatial structure (Fig. 14). Of the 34 species, only 12 were distributed throughout the main body of the lake. The remaining 22 species were found exclusively in restricted areas near the river inflows. They enter the system, but for some reason do not spread throughout it. The points of entry of these species are clearly identifiable: *D. sicilis* enters from the Precambrian Shield drainage areas via the Berens, Mukatawa, and Poplar rivers, *D. longiremis* and *D. minutus* come solely from the Saskatchewan River, and *D. siciloides* and *D. oregonensis* are brought by rivers flowing from the sedimentary basin, i.e. the Saskatchewan, Warpath, and Red rivers. *Mesocyclops edax* invades the lake in very small numbers in the autumn from three major rivers, the Saskatchewan, Red, and Winnipeg. *Chydorus sphaericus* enters from the northwest but surprisingly is not found in the vicinity of the Red River inflow even though it is considered to be one of the most widespread cladocerans. *Holopedium gibberum*, one of the most widely distributed cladocerans in Canada, was found only once in very small numbers near the Winnipeg River inflow. Several daphnids, *D. schoedleri, D. pulex, D. ambigua, D. parvula,* and *C. quadrangula*, arrive only by the Red River and do not expand beyond the river inflow area.

Thus, one can distinguish two groups in the planktonic community, a "core" of 12 established species and the remaining 22 "marginal," unsuccessful invaders (Patalas 1981). It seems possible that the marginal species might constitute a potential replacement pool if some core components were eliminated by changing environmental conditions. Such a planktonic community structure could ensure quick adaptation of the community to changing abiotic and biotic conditions.

The perception of the complexity of a system is a function of the level of resolution applied in the observation. This general statement is particularly illustrated in the analysis of the crustacean plankton of Lake Winnipeg. When the resolution is limited to seasonal mean total crustacean abundance, only small differences between the North and South basins are found (50 and 55 ind.·L^{-1}). Greater differences, are, however, visible when the seasonal and geographic dimensions are examined. South Basin plankton abundances appear to increase much faster than those in the North Basin (Fig. 5). After the total crustacean plankton was subdivided into three major taxonomic

FIG. 12. Spatial distribution of (ind.·L^{-1}) of adults and juveniles of (a) *Ceriodaphnia quadrangula*, (b) *Bosmina longirostris*, and (c) *Chydorus sphaericus* in Lake Winnipeg, June–October 1969.

FIG. 13. Spatial distribution of (ind.·L^{-1}) of adults and juveniles of (a) *Leptodora kindtii*, (b) *Diaphanosoma leuchtenbergianum*, and (c) total planktonic crustaceans in Lake Winnipeg, June–October 1969.

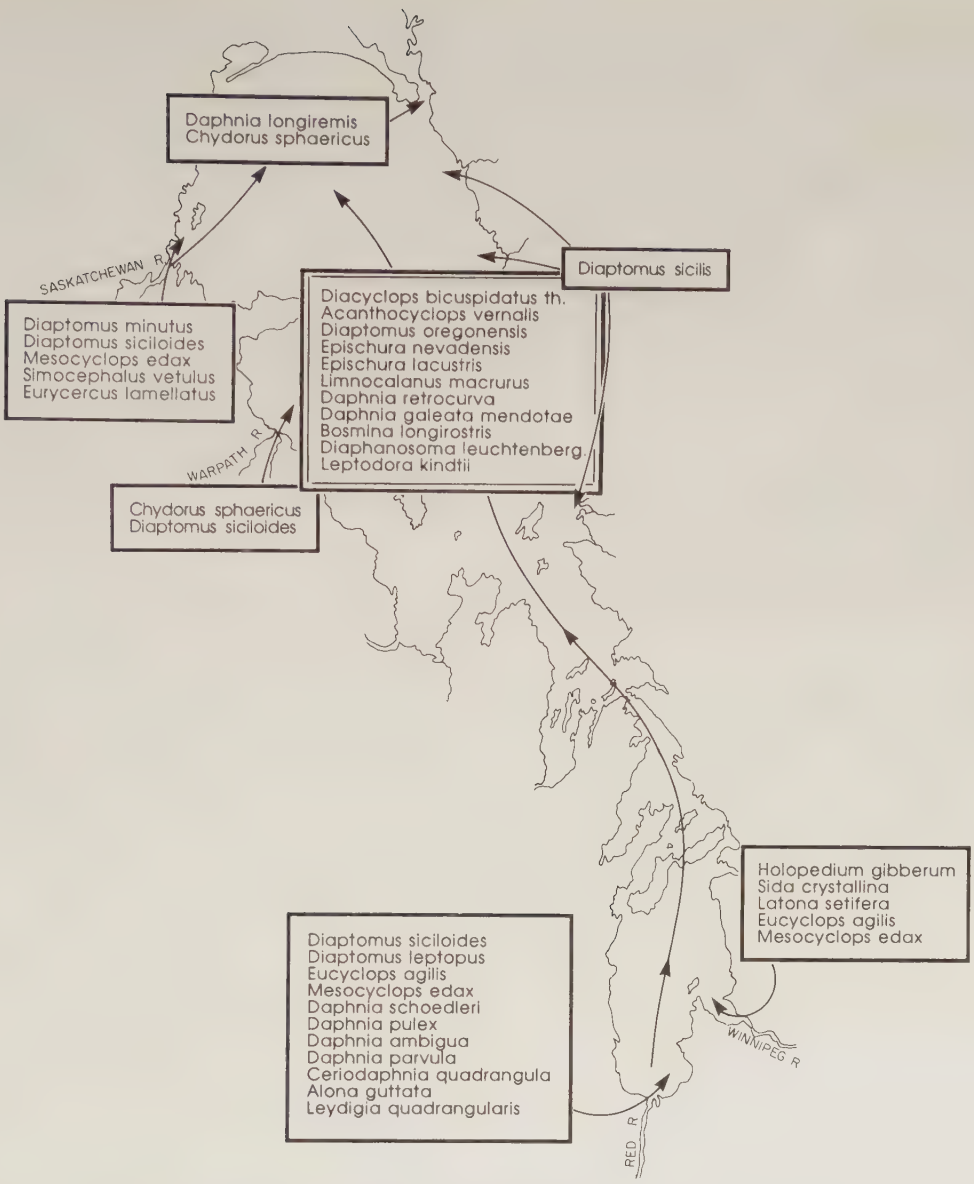

FIG. 14. Schematic diagram of two components of the crustacean community in Lake Winnipeg, "core" species (doubled framed), established all over the lake, and "marginal," unsuccessful invaders (single framed) and their routes of entry.

groups, differences between basins increased substantially. The ratio of cladocerans to calanoids to cyclopoids was 1:6:3 in the South Basin and 1:3:8 in the North Basin, respectively, showing a basically different community structure. Calanoids dominated the South Basin, while cyclopoids did in the North Basin. Cladocerans were less abundant and contributed only 8–10% to the total abundance of crustaceans in both basins (Fig. 4).

Increasing resolution to the individual species level reveals much larger interbasin differences (Table 3). Only 15 of the 34 species found from June to October were common to both basins. Expressing this ratio in the form of a coefficient of community (Jaccard 1912):

$$\frac{c}{c + a + b} = 0.44$$

where a = number of species found only in the North Basin (7), b = number of species found only in the South Basin (12), and c = number of species common to both basins.

As Jaccard's coefficient of community is based on the presence or absence of species, it does not reflect the quantitative relationships between the species in the two compared samples. The percentage similarity index (Renkonen 1938)

$$Pij = \Sigma\text{Min}\ \{xki,\ xkj\}$$

places emphasis on dominant species and is calculated as the sum of the smaller percentage of each species within a sample pair of i,j. This means that in Jaccard's coefficient, every species has the same "weight," and in percentage similarity by Renkonen, the "weight" of the species is proportional to its relative abundance. Both indices range from 0 in two samples without a single common species to 1 where two samples have all the same species, and, as in Renkonen's percentage similarity, those species occur in the same proportions. Within a well-mixed water mass, the distribution of planktonic animals would be expected to be relatively uniform, and both similarity indexes between samples would tend to reach a value of 1.0.

Renkonen's percentage similarity index calculated for the North and South basins amounted to a rather low mean value of 0.60, ranging seasonally from 0.42 to 0.76. This indicates that not only the overall species composition, as shown by Jaccard's community coefficient, but also the proportions of dominant species varied substantially between the basins. Low similarity shown by both indices reinforces the conclusion about the profound differences existing in the structure of the plankton between the North and South basins.

Spatial variation in plankton composition and abundance existed also within each basin although it was expected to be smaller than between the basins. In order to measure the spatial variance, four regions were defined within each basin. The division was based on the morphology of the lake and the location of the major inflows (Fig. 1). Percentage similarities (Renkonen 1938) were then calculated between all pairs of regions and the results for five consecutive months presented in the form of dendrograms (Fig. 15). A complete linkage agglomerative clustering analysis (Sørensen 1948) was used. In this method, two clusters can fuse only when all objects of the first are linked to all objects of the second (Legendre and Legendre 1983).

The following conclusions are drawn from these dendrograms and the maps of distributions of the individual species (Fig. 6a–13b):

(1) Within the North Basin, the plankton communities of the Saskatchewan River vicinity and the northern part of the North Basin were most similar. Their similarity index was 0.87 on average, ranging from 0.68 to 0.94 during the period from June to October. The eastern part of the North Basin was least similar to the other regions, average similarity with the Saskatchewan River vicinity, the northern part of the North Basin, and the western part of the North Basin being 0.52, 0.55, and 0.66, respectively.

(2) Within the South Basin, the Red River vicinity and main part of South Basin were the most similar areas at a level averaging 0.80 and ranging from 0.68 to 0.92. Plankton of the Winnipeg River vicinity was very different from the neighbouring South Basin region, with similarity values not exceeding 0.47. These values indicate that although the Red River inflow supplies only 20% of the water, it is rich in plankton and it has a stronger influence on the character of the planktonic communities in the downstream portion of the basin than the Winnipeg River which supplies 80% of the water (Brunskill et al. 1980) but is extremely poor in plankton. Thus, in spite of a different plankton composition, the Winnipeg River has mainly a diluting effect on the downstream portion of the basin. The geographic proximity of two lake regions, therefore, is not always reflected in a higher similarity of their plankton.

(3) The two pairs of most similar regions (Saskatchewan River vicinity – northern part of the North Basin and Red River vicinity – South Basin) are linked at a rather low level of 0.42 (0.24–0.66) similarity. They are situated at the two extreme north and south ends of the lake, which are subject to differences in climate and fed by different rivers, the Saskatchewan River draining the northern portion of the Saskatchewan prairies with many lakes and reservoirs and the Red River flowing from North Dakota with very few lakes.

(4) Plankton of the Narrows is intermediate in character with an average similarity of 0.68 with both the eastern part of the North Basin and the South Basin. In four of five months, however, it exhibited a tendency to cluster with the South Basin.

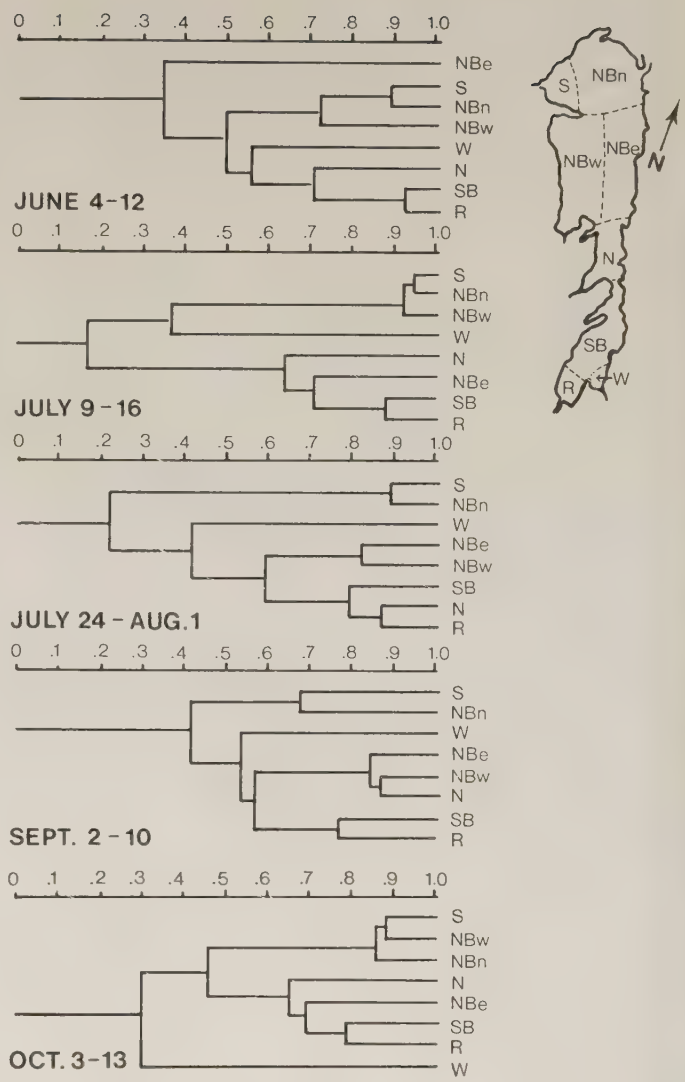

FIG. 15. Seasonal changes in plankton similarity (Renkonen 1938) between various regions of Lake Winnipeg (complete linkage agglomerative clustering; Sørensen 1948) (R, Red River; W, Winnipeg River; SB, main part of the South Basin; N, Narrows; NB, North Basin with subdivisions e (east), w (west), and n (north); S, Saskatchewan River).

(5) The similarity of plankton among the inflow areas of the three major rivers was low. On average, Winnipeg River plankton exhibited similarity indices of only 0.44 and 0.49 with the Saskatchewan and Red rivers, respectively. The Red River inflow area was more similar to the remote Saskatchewan River inflow area (0.55) than to the closely situated Winnipeg River area (0.49). Both the Saskatchewan and Red rivers, although different in character, drain the sedimentary, prairie area, while the Winnipeg River supplies water from the Precambrian Shield.

(6) Plankton in the eastern part of the North Basin was very different from that in other regions in June (similarity 0.33). Water temperatures were 2–3°C lower than in the remaining parts of the lake (Brunskill et al. 1979a), causing a delay in planktonic development. In July, with more water flowing from the South Basin, the plankton of the eastern part of the North Basin became more similar to that in the South Basin (similarity 0.70). In August, September, and October, plankton in all four

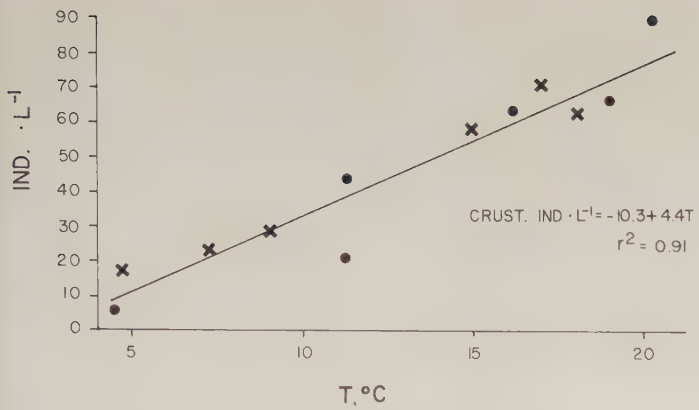

FIG. 16. Relationship between mean total number of planktonic crustaceans and the mean temperature of water in the upper 10-m layer in Lake Winnipeg during June–October 1969. Dots and crosses denote South and North basins, respectively.

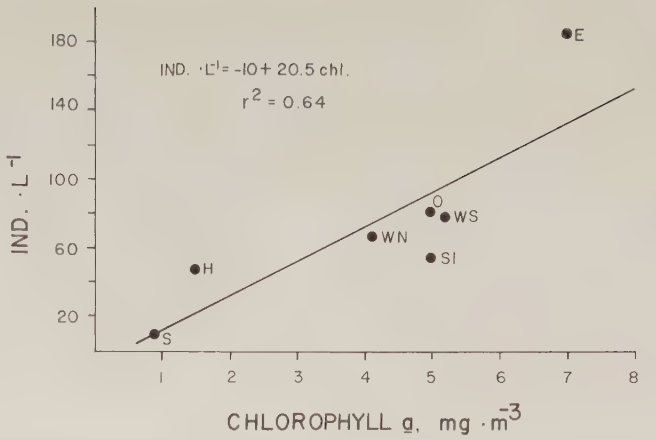

FIG. 17. Relationship between mean crustacean plankton abundance and chlorophyll *a* concentration in several North American great lakes (E, Lake Erie; O, Lake Ontario; WS, Lake Winnipeg South; WN, Lake Winnipeg North; SI, Southern Indian Lake; H, Lake Huron; S, Lake Superior). Chlorophyll a data from Vollenweider (1976), Brunskill et al. (1979a), Hecky (1975).

regions of the North Basin were more similar to each other than to regions in the South basin.

In general, dendrogram analysis confirmed the higher similarity of plankton between regions within the same basin (usually within the range 0.75–0.95) than between the North and South basins, where the similarity was 0.45 (range 0.24–0.60). Exceptions were regions fed by water from the Precambrian Shield, i.e. the Winnipeg River inflow area in the South Basin and the eastern part of the North Basin.

Water temperature seems to be one of the main factors explaining plankton abundance and its distribution in Lake Winnipeg. On average, water temperatures were 1.77°C lower in the North Basin than in the South Basin. If morphometry were responsible for the temperature differences, then the South Basin would be expected to warm up earlier in the spring and cool earlier in the autumn than the deeper, larger North Basin. Instead, in both parts of the lake, water temperatures closely followed air temperatures with a 6- to 12-d lag. The North Basin cooled earlier, indicating that climate was a more important factor than basin morphology.

Seasonal changes in crustacean abundance were also apparently related to water temperature (Fig. 16). Total numbers of crustaceans increased with increasing temperatures, reaching a maximum between late July and early September. Much higher numbers of plankters were found in spring in the warmer South Basin than in the colder North Basin. Cooling of water in October was followed by a decrease in plankton abundance in both the North and South basins. A significant correlation existed between planktonic crustacean abundance and epilimnion temperature (Fig. 16). Such a correlation could not be expected in temperate lakes where spring and autumn maxima are often separated by a midsummer minimum. In Lake Winnipeg, however, there is little indication of seasonal bimodality. Only in the North Basin does some weak indication of such a bimodality exist in chlorophyll concentration (Fig. 5) (see also Hecky et al. 1986). There is a certain minimum response time necessary between a chlorophyll maximum and a succeeding zooplankton peak. Only Cyclopidae nauplii and *B. longirostris*, the two smallest and probably fastest growing categories, responded to the spring and late summer chlorophyll maxima. The remaining species produced rather single-mode patterns, probably because of a shorter ice-free season and the relatively low temperatures prevailing in the lake. Temperatures above 17°C do not occur for longer than 4–6 wk, and during the remaining period, temperature could be considered as the factor limiting zooplankton growth and reproduction.

Although no correlation was found between chlorophyll *a* and crustacean abundance, the seasonal pattern of both parameters displayed some similarity (Fig. 5). In the South Basin, maximum concentrations of chlorophyll *a* were found in mid-July, 2 wk before a maximum of crustacean abundance occurred (G. J. Brunskill, unpubl. data). The September chlorophyll *a* maximum in the North Basin coincided with the maximum of planktonic crustaceans.

A seasonal succession of species caused by differences in environmental requirements and possibly by interspecific competition was quite apparent in both the South and North basins (Fig. 5). The mid-July peak of *D. retrocurva* and *B. longirostris* in the South Basin was followed by the late July maximum of *D. leuchtenbergianum* and *L. kindtii* and a September maximum of *D. galeata mendotae*. A similar succession, although delayed by about 2–4 wk, was observed in the North Basin. The June maximum of *D. bicuspidatus thomasi* in the South Basin was followed by an *A. vernalis* maximum in September, with a similar succession, delayed by 1 mo, in the North Basin. The *Epischura* spp. and *D. ashlandi* late July peaks were succeeded by a *D. siciloides* maximum in September and an October peak of *D. oregonensis*. Delays in population maxima in the North Basin coincided with climate-related, cooler temperatures in this part of the lake, particularly in late spring and early summer, and also with a delayed chlorophyll *a* maximum (Fig. 5).

The average number of planktonic crustaceans in midsummer samples from the South and North basins amounted to 90 and 63 ind.·L^{-1}, respectively. These figures are relatively high when compared with other great lake plankton densities (Patalas 1975). Lake Winnipeg plankton abundances of 63–90 ind.·L^{-1} fall into the upper middle portion of the range for North American great lakes (Table 4). Only Lake Erie had more crustaceans per litre than Lake Winnipeg. No significant correlation was found between crustacean abundance, mean depth, and Secchi disc transparency. However, as demonstrated (Patalas 1975), in such a wide range of lakes, crustacean abun-

TABLE 4. Midsummer abundance of crustaceans and some limnological characteristics of several North American great lakes. (Midsummer values of the whole water column or 0-50 m in deeper lakes. Standard errors in parentheses. The limnological characteristics are based mostly on Rawson (1960), Chandler (1964), Brunskill et al. (1979a, 1979b, 1980), Patalas and Patalas (1978), Patalas (1969), and on the data from the Canada Centre for Inland Waters (Anonymous 1970a, 1970b, 1970c). Well-separated basins of Lake Erie, Lake Winnipeg, and Great Slave Lake were treated here as distinct water bodies.

Lake	Mean depth (m)	Secchi (m)	Epilimnion temperature mean (°C)	Crustaceans (ind.·L^{-1})
Erie Central	18	2.9 (0.25)	21.7 (0.30)	184 (26.8)
Erie East	24	3.6 (0.41)	21.0 (0.50)	154 (27.1)
Erie West	8	2.0	23.5 (0.10)	128 (12.8)
Ontario	86	3.2 (0.16)	18.5 (0.60)	80 (10.4)
Winnipeg South	9	0.8	20.3 (0.22)	90 (16.3)
Winnipeg North	12	1.9	18.5 (0.19)	63 (5.7)
South Indian	9	2.0 (0.09)	17.5 (0.13)	54 (5.4)
Reindeer	17	7.0 (0.21)	14.2 (0.11)	50 (3.2)
Huron	59	7.1 (0.29)	17.6 (0.40)	48 (5.1)
La Martre	11	6.0	12.8 (0.22)	21 (2.2)
Superior	148	7.9 (0.27)	9.5 (0.38)	10 (0.9)
Great Slave West	41	2.5 (0.30)	10.0 (0.62)	3 (0.3)
Great Slave East	185	9.0 (0.60)	4.0 (0.40)	3 (0.3)
Great Bear	76	20.0	4.0	1

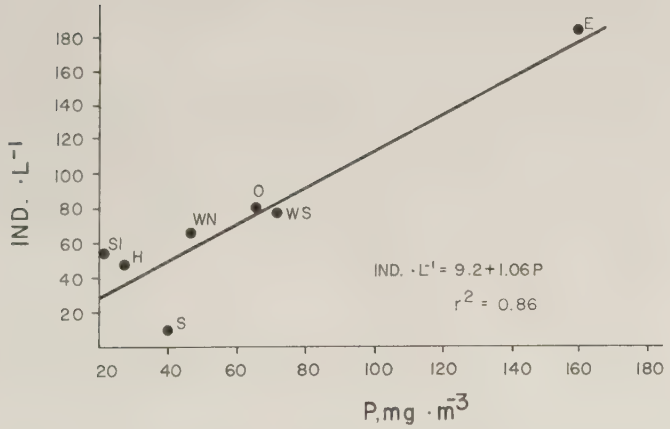

FIG. 18. Relationship between mean crustacean plankton abundance and mean concentration of total phosphorus in inflowing waters (E, Lake Erie; O, Lake Ontario; WS, Lake Winnipeg South; WN, Lake Winnipeg North; SI, Southern Indian Lake; H, Lake Huron; S, Lake Superior). Phosphorus data from Vollenweider (1976), Brunskill (1974), and Hecky (1975).

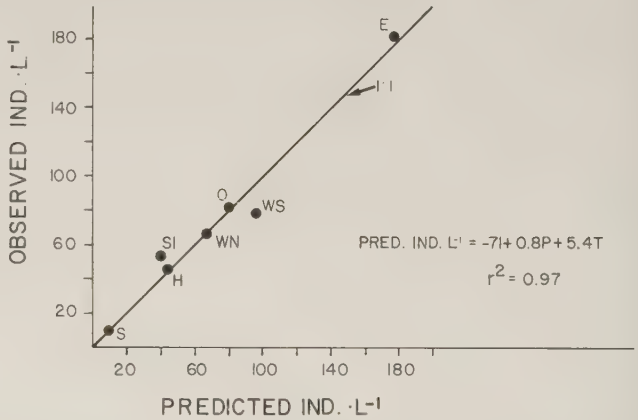

FIG. 19. Planktonic crustacean abundance observed and predicted from the mean concentration of total phosphorus in the inflowing waters and epilimnion temperature in summer (E, Lake Erie; O, Lake Ontario; WS, Lake Winnipeg South; WN, Lake Winnipeg North; SI, Southern Indian Lake; H, Lake Huron; S, Lake Superior).

dance was significantly correlated with midsummer epilimnion temperature ($r^2 = 0.92$; $p < 0.01$). The correlation with chlorophyll *a* concentration (Fig. 17) was significant but only barely so ($r^2 = 0.64$; $p = 0.05$).

A good correlation ($r^2 = 0.86$; $p < 0.01$) existed between midsummer crustacean density and annual mean total phosphorus concentration in inflowing water (Fig. 18). Only very cold Lake Superior revealed crustacean numbers lower than predicted from the regression. A multiple regression with epilimnion temperature and phosphorus concentration in inflowing water used as independent variables (Fig. 19) gave a much better correlation ($r^2 = 0.97$; $p < 0.01$).

Crustacean densities observed in the North Basin of Lake Winnipeg were very close to those expected. Values in the South Basin, about 20% lower than predicted, may have been influenced by apparent light limitation (Brunskill et al. 1979b; Healey and Hendzel 1980) which prevented full utilization of available nutrients for algal production. As indirect evidence, the average concentration of chlorophyll *a*, 5.3 mg·m^{-3}, observed in the South Basin is much lower than the 14.8 mg·m^{-3} predicted from phosphorus inflow concentration (Pi) and water renewal time (*t*) using Vollenweider's (1976) formula:

$$\text{Chlorophyll } a = 0.35\ \text{Pi} \cdot \frac{1}{1 + \sqrt{t}},$$

$$\text{Pi} = 72\ \text{mg}\cdot\text{m}^{-3} \text{ and } t = 0.5\ \text{yr}.$$

Has the plankton of Lake Winnipeg changed in response to cultural eutrophication within its drainage basin? One of the earliest and most complete studies of plankton composition and abundance in Lake Winnipeg was conducted by Bajkov (1930, 1934). Of the 34 species recorded in the present study, 19 were previously recorded by Bajkov (Table 5). Sixteen littoral and benthic species listed by Bajkov were not recorded in 1969 mainly due to the pelagic nature of sampling sites in this study.

TABLE 5. List of species found in Lake Winnipeg in 1929 as recorded by Bajkov (1934) and in 1969 by the present authors.

1929	1969
Limnocalanus macrurus Sars	*Limnocalanus macrurus* Sars
Epischura lacustris Forbes	*Epischura lacustris* Forbes
	Epischura nevadensis Lilljeb.
Diaptomus ashlandi Marsh	*Diaptomus ashlandi* Marsh
	Diaptomus oregonensis Lilljeb.
Diaptomus sicilis Forbes	*Diaptomus sicilis* Forbes
	Diaptomus siciloides Lilljeb.
	Diaptomus minutus Lilljeb.
Diaptomus leptopus Forbes	*Diaptomus leptopus* Forbes
	Diaptomus clavipes Schacht
Cyclops bicuspidatus Claus	*Diacyclops bicuspidatus thom.* Forbes
	Acanthocyclops vernalis (Fischer)
Cyclops viridis Jurine	
Cyclops prasinus Fischer	*Eucyclops agilis* (Koch)
Cyclops phaleratus Koch	*Mesocyclops edax* (Forbes)
Cyclops serrulatus Fischer	*Macrocyclops albidus* (Jurine)
Cyclops fimbriatus Fischer	*Daphnia retrocurva* Forbes
Daphnia retrocurva Forbes	
Daphnia longispina Mueller	
Daphnia longispina hyalina Leydig	*Daphnia galeata mendotae* Birge
	Daphnia pulex Leydig
Daphnia pulex de Geer	*Daphnia schoedleri* Sars
	Daphnia longiremis Sars
Ceriodaphnia pulchella Sars	*Daphnia parvula* Fordyce
	Daphnia ambigua Scourfield
Simocephalus vetulus Mueller	
Simocephalus serrulatus Koch	*Ceriodaphnia quadrangula* (Mueller)
Moina rectirostris (Leydig)	*Simocephalus vetulus* Schoedler
Bosmina longirostris (Mueller)	
Bosmina longispina Leydig	*Bosmina longirostris* (Mueller)
Chydorus sphaericus (Mueller)	
Chydorus gibbus Lilljeb.	*Chydorus sphaericus* (Mueller)
Alona affinis Leydig	
Alona costata Sars	*Alona guttata* Sars
	Alona quadrangularis (Mueller)
Pleuroxus procurvatus Birge	*Leydigia quadrangularis* (Leydig)
Pleuroxus denticulatus Birge	*Latona setifera* (Mueller)
Kurzia latissima (Kurz)	
Eurycercus lamellatus (Mueller)	*Eurycercus lamellatus* (Mueller)
	Holopedium gibberum Zaddach
Diaphanosoma brachyurum (Lievin)	
Diaphanosoma leuchtenbergianum (Fisch.)	*Diaphanosoma leuchtenbergianum* (Fischer)
Sida crystallina (Mueller)	*Sida crystallina* (Mueller)
Leptodora kindtii (Focke)	*Leptodora kindtii* (Focke)

The remaining 15 species, not recorded by Bajkov, mostly very rare, were found in the present study only in restricted areas. Species more widely distributed in 1969 but not mentioned by Bajkov are *E. nevadensis*, *D. oregonensis*, *D. siciloides*, *A. vernalis*, and *D. longiremis*.

Epischura nevadensis is difficult to distinguish from *E. lacustris* even in late copepodid stages. *Cyclops viridis* listed by Bajkov is probably *A. vernalis*. Yeatman (1959) mentioned that most specimens recorded as *C. viridis* are really other species, mainly *A. vernalis*. Changes in taxonomy could certainly explain the discrepancies between Bajkov's *Daphnia* list and that of the present authors (Brooks 1957; Wilson 1959).

Thus, it is unlikely that any of the planktonic species recorded by Bajkov in 1929 (Bajkov 1934) disappeared from the lake by 1969. The increased number of species recorded in the 1969 samples is probably due to the increased intensity of sampling and to some extent to the taxonomy applied. By applying the settling procedure described in Bajkov (1930), a comparison of the settled net plankton volumes (including both phyto- and zooplankton) in 1929 and 1969 was possible. Lake Winnipeg settled net plankton volumes during the 1929 season were significantly below 1969 values except for mid-August data when the density was 20.2 $mm^3 \cdot L^{-1}$, about five times that of the other summer months. Unfortunately, no sampling was done on a comparable date in August 1969 during the 5-wk interval from July 27 to September 4, and an algal bloom could have been missed. In spite of this, the 1969 seasonal average settled net plankton volume was almost double that in 1929, 9.2 and 5.8 $mm^3 \cdot L^{-1}$, respectively. When the August 1929 extreme peak is excluded, the average 1969 net plankton is three times greater than in 1929, 9.2 and 3.0 $mm^3 \cdot L^{-1}$, respectively.

Secchi disc transparency can be another indication of changing conditions, but because it varies with season and location, it would be difficult to document statistically significant changes between 1929 and 1969. However, cautious examination of the existing data suggests that some significant changes may have occurred in the lake during this 40-yr period. Bajkov (1930) recorded 1.5-m Secchi disc transparency in the middle of the South Basin in 1929. The corresponding 1969 average measurement at stations 57 and 59 from July until September was 0.74 m, with a range of 0.5–1.0 m. The North Basin exhibited a reversed trend with a Secchi depth of 1.6 m in 1929 and 2.2 m in 1969. Also, near the mouth of the Saskatchewan River, a transparency of only 0.1 m was recorded in 1929, but as much as 2.1 m (range 1.2–3.0 m) was found, on average, between July and September 1969 (Brunskill et al.

TABLE 6. Monthly mean air temperatures in the summers of 1929 and 1969 (Transport Canada, Meteorological Branch).

Month	Temp. (°C) 1929	1969	Difference between 1929 and 1969 (°C)
June	17.0	12.2	+ 4.8
July	20.1	18.5	+ 1.1
August	20.2	20.9	−0.7
Mean	19.1	17.2	+ 1.9

1979a). The latter values have also been confirmed by Derksen and Hangasjarvi (1979) who recorded 2.4 m on average, with a range of 1.5–3.0 m, in July 1974 in the same area. Not a single 1969 measurement was close to the 0.1-m Secchi depth measured in 1929. The higher North Basin transparencies in 1969 could be explained by the Cedar Lake – Grand Rapids impoundment completed in about 1964. This new reservoir could act as a sediment and nutrient trap for the Saskatchewan River. On the other hand, nutrient loading rates to the South Basin have probably increased since 1929. Although there was a lack of direct measurement in 1929, some extrapolation can be made from later (Brunskill 1974; Brunskill et al. 1980) estimates. According to these authors, the 1970–74 loading of total phosphorus, expressed as the annual mean concentration in water flowing into the South Basin of Lake Winnipeg, was 97 $mg \cdot m^{-3}$.

A hypothetical phosphorus loading for 1929 can be back-calculated using the following assumptions: (1) the human and livestock population in 1929 amounted to 0.6–0.7 of 1970 levels, (2) the use of fertilizers in 1929 was not greater than 0.05 of the 1970 level, and (3) the anthropogenic input in 1970 was 0.7 of the total phosphorus loading.

The 1929 total phosphorus loading estimate, based on these assumptions, was 50 $mg \cdot m^{-3}$, i.e. about half the 1970 level. Similarly, during the period from 1929 and 1969 the net plankton settled volume in Lake Winnipeg doubled. The average Secchi disc transparency of water in the South Basin decreased from 1.5 to 0.75 m but increased in the North Basin, probably due to the sediment and nutrient trapping effect in Cedar Lake.

It is difficult to conclude with certainty whether these differences represent normal, climate-related, year-to-year variation rather than effects of increased nutrient loading. Comparison of the climatic conditions in these two years (Table 6) indicates that two of three months in the summer of 1929 were warmer than in 1969, and the corresponding mean temperatures were on average 1.9°C higher than in 1969. Plankton abundance usually increases with temperature in lakes of the temperate zone (Fig. 16; see also Patalas 1975). With nutrient loading constant, greater amounts of plankton are observed in warmer summers. Thus, in Lake Winnipeg, because more plankton was found in 1969 in spite of the cooler summer, the increased abundance of plankton appears to be a function of increased nutrient loading.

Most of the conclusions in this paper could not have been reached without the application of maps of distribution of individual species. These maps allowed an analysis of invasion routes and changes in the distribution in consecutive months indicating rates and directions of expansion. This analysis of individual species behaviour sheds some light on the formation and dynamics of the planktonic community in an aquatic system as large and complex as Lake Winnipeg. Also, it helped us to understand the response of planktonic community structure to abiotic factors in the ecosystem. Patterns as complex as those in Lake Winnipeg were also observed in other North American great lakes although the causes of such a complexity may differ in each individual case (Patalas 1990).

This study demonstrated that lake morphology, the location of major river inflows, the geology of the drainage basin, and the climatic differences within the system sufficiently explained most of observed patterns of distribution of planktonic crustaceans.

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Zooplankton in Advective Environments: The Hudson River Community and a Comparative Analysis

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The temporal dynamics and spatial distributions of zooplankton in the tidal freshwater portion of the Hudson River were studied over a 3-yr period. We tested the hypothesis that advective transport regulates zooplankton biomass in the Hudson and in lakes, estuaries, and rivers for which we have published values. In the Hudson, zooplankton biomass was negatively correlated with discharge over the entire season ($P < 0.0001$) as well as during the warmer period of the year ($P = 0.007$) when biomass was greatest. The spatial distribution of zooplankton over 160-km transects was heterogeneous. Downstream changes in the abundance of a dominant species, *Bosmina longirostris*, indicate that certain areas of the river support net population growth whereas other areas are population sinks. We infer that zooplankton biomass in the Hudson is a function of the balance between reproduction determined by resources and losses due to advection. Zooplankton biomass differs among lakes, estuaries, and rivers in a manner consistent with the differences in water residence time. Biomass is highest in lakes, lower in saline estuaries and tidal rivers, and lowest in rivers. Advective losses appear to be important in explaining differences between planktonic communities in lentic and lotic environments.

La présente étude triennale a porté sur la dynamique temporelle et la répartition spatiale du zooplancton retrouvé dans le tronçon d'eau douce à marée du fleuve Hudson. Les auteurs ont vérifié l'hypothèse selon laquelle le transport advectif règle la biomasse zooplanctonique dans l'Hudson et dans des lacs, estuaires et cours d'eau déjà étudiés. Ils ont déterminé que la biomasse zooplanctonique de l'Hudson est en corrélation négative avec le débit pendant toute la saison ($P < 0{,}0001$), ainsi que pendant la période plus chaude de l'année ($P = 0{,}007$) lorsqu'elle était à son maximum. La répartition spatiale du zooplancton dans des transects de 160 km était hétérogène. Les variations de l'abondance de *Bosmina longirostris*, l'espèce dominante, dans les eaux d'aval révèlent que certains tronçons du fleuve favorisent une croissance nette des effectifs, tandis que d'autres tronçons agissent comme puits. Les auteurs en déduisent que la biomasse zooplanctonique de l'Hudson est fonction de l'équilibre entre, d'une part, la reproduction telle que déterminée par les ressources disponibles et, d'autre part, les pertes par advection. La biomasse zooplanctonique varie selon les lacs, estuaires et cours d'eau de façon compatible avec les différences du temps de séjour des eaux. Cette biomasse est plus élevée dans les lacs, intermédiaire dans les estuaires d'eau salée et les cours d'eau à marée et plus faible dans les autres types de cours d'eau. Les pertes par advection semblent être un facteur important pour expliquer les différences entre les communautés planctoniques de milieux lénitiques et lotiques.

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Studies of aquatic populations, particularly in marine systems, have documented the importance of hydrodynamics in determining spatial and temporal patterns of abundance (Legendre and Demers 1984). Physical transport of organisms may move populations away from areas suitable for growth, while circulatory features which promote retention may enhance population growth. These mechanisms may be substantially more important than density-dependent factors such as resource or space limitation in controlling marine populations (Roughgarden et al. 1988; Sinclair 1988). Generally, studies of the ecological effects of hydrological transport have focused on populations and have not considered the broader question of whether entire communities may be influenced by transport. The biomass of a community may also be limited by high advective transport. If transport is important, community biomass should be higher when advection is low. In considering different aquatic ecosystems, one could test this idea by comparing community biomass in environments with high advective transport relative to environments with low advective transport.

The dynamics of river zooplankton are often considered to be controlled by transport of populations (Hynes 1970). Recent studies confirm this view while also documenting significant population growth during transport (Saunders and Lewis 1989). In addition, other factors such as turbidity and food may regulate zooplankton in rivers. High concentrations of suspended organic and inorganic particles can have negative effects on the ingestion, growth, and reproduction of certain zooplankton (Arruda et al. 1983; McCabe and O'Brien 1983; Threlkeld 1986; Hart 1987, 1988; Kirk and Gilbert 1990). Further, although phytoplankton and bacteria may be abundant in rivers, a substantial portion of these resources may be either inedible, toxic, or poor-quality food (Arruda et al. 1983; Pace et al. 1983; Fulton and Pearl 1987; Hart 1988).

Zooplankton in the tidal freshwater portion of rivers are a particularly important component of food webs. Anadromous and resident fishes in tidal rivers feed on zooplankton during early life history stages. For example, the commercially important, anadromous species *Morone saxatilis* develops in the freshwater and brackish portion of estuaries along the eastern

seaboard of the United States and Canada (Smith 1985). In the Potomac River, *M. saxatilis* larvae feed selectively on freshwater cladocerans (Setzler-Hamilton et al. 1981). Furthermore, there are significant correlations between the nutritional condition of *M. saxatilis* larvae and the abundance of cladocerans and copepods in the Potomac (Martin et al. 1985). This example and other case studies (Crecco and Blake 1983; Stevens et al. 1987) indicate that tidal freshwater zooplankton are a key trophic linkage between microorganisms (bacteria, phytoplankton, and protozoans) and fish.

Despite the significance of zooplankton in tidal freshwater ecosystems, there have been few studies of their dynamics and little consideration of the factors limiting these communities. We describe here the spatial and temporal dynamics of zooplankton in the tidal freshwater portion of the Hudson River. The significance of potential regulatory factors including temperature, food, and advection is evaluated for a 3-yr sampling series from a single station and a less frequently sampled, spatially distributed set of stations.

We then consider the hypothesis that zooplankton biomass in tidal rivers and saline estuaries exceeds biomass found in nontidal rivers but is lower than zooplankton biomass in lakes. The proposed mechanism underlying this hypothesis is based on a balance between the net growth of populations in the system and advective removal of populations. As presented by Ketchum (1954), net population growth within an estuary must exceed the loss of biomass in water exchanged on each tidal cycle. In the upper reaches of most rivers, downstream advection is so rapid that planktonic populations cannot develop (Hynes 1970). In downstream reaches or in tidal portions, positive population growth is possible due to greater water residence time. The conceptual model, in this case, is of a system with continuous inputs and outputs of water, where inputs contain no or few zooplankton and outputs are a function of the biomass of zooplankton in the system and water flow. Thus, the biomass of zooplankton observed within a system will be a function of the net production of biomass minus advective removal. If net production of biomass is largely a function of food availability and does not vary systematically across lakes, rivers, and estuaries, then biomass of zooplankton in these systems will, we propose, be related to differences in advection. In addition, we recognize the possibility that zooplankton biomass may be higher in the saline portion of estuaries as a consequence of opposing currents in stratified estuaries which in combination with vertical migrations position the organisms to be moved upstream on flooding tides and retained during ebbing tides (Cronin 1982; Cronin and Forward 1986; Kennish 1990). This mechanism, if important, would tend to lower downstream advective losses in stratified estuaries and result in higher community biomass relative to unstratified estuaries. These arguments suggest overall that lakes with little advective removal will have the highest zooplankton biomass, followed by stratified estuaries, tidal rivers, and nontidal rivers. To test this hypothesis, we compare estimates of mean biomass from published studies of these systems.

Methods

Study Site

This study was conducted in the tidal portion of the Hudson River. A series of stations along the north–south axis of the river was sampled (Fig. 1). The tidal amplitude over this distance averages about 1 m. We measured conductivity at each station. The intrusion of seawater (defined as salinities >1.0 ppt) ranged only into the two most seaward stations

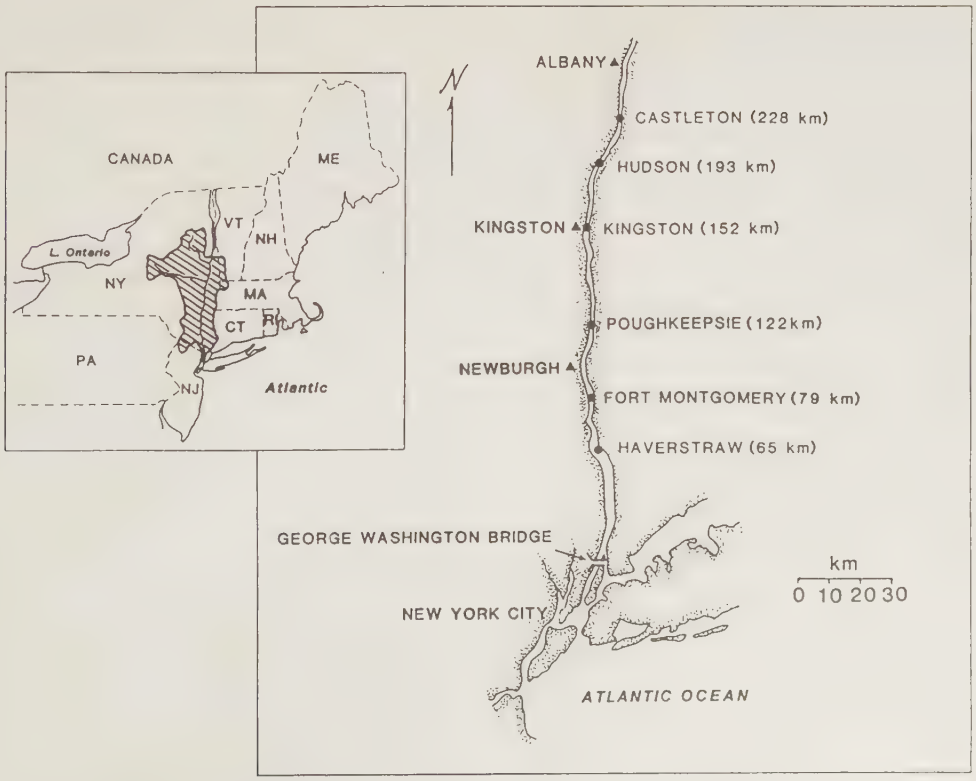

FIG. 1. Map of the Hudson River showing sampling stations and their distance from the mouth of the river. River km 0 is at Battery on the southern tip of Manhatten Island, New York.

during late summer. The maximum salinity we observed was 11 ppt during late summer at Haverstraw Bay (Fig. 1). Our study region was, therefore, primarily the freshwater section of the estuary except in late summer when the oligohaline portion was also included in the studies of spatial distribution (see below). It is important to note that, in the region of the Hudson River we studied, the water column is uniformly mixed so that there is no vertical stratification in temperature or salinity.

Tidal flow dominates in the Hudson. Freshwater input into the estuary arises primarily from water flowing over a dam at Green Island, New York. Mean freshwater flow is 400 $m^3 \cdot s^{-1}$ at Green Island whereas mean tidal flow at the mouth of the Hudson is 12 000 $m^3 \cdot s^{-1}$ (Moran and Limburg 1986; Cooper et al. 1988). Average residence time of water within the estuary is 126 d, but residence time varies seasonally with freshwater flow. Highest inputs of freshwater occur in the spring (March and April). Lowest inputs are in August and September (Cooper et al. 1988).

Sampling

A temporal sampling series was conducted at a freshwater station near Kingston, NY (river km 152), during 1987–89 (Fig. 1). This station was visited once every 2 wk between April and December. A less frequent spatial sampling series was conducted along the axis of the river at six stations, ranging from Castleton, NY (river km 228), to Haverstraw Bay (river km 65) (Fig. 1). These longitudinal surveys were conducted in the fall of 1987 ($n = 2$), monthly between April and November 1988 ($n = 8$), and four times (April, June, August, and October) in 1989.

Zooplankton were sampled at 0.5 m depth with an open diaphragm bilge pump. Because the river is uniformly mixed, a sample from a single depth is sufficient to characterize the water column (Findlay et al. 1991a, 1991b). For macrozooplankton, triplicate 105-L samples were taken and concentrated by passing the outflow of the pump through a 70- to 80-μm-mesh net, except in 1987 when a 150-μm-mesh net was used. Triplicate 2-L samples for microzooplankton were collected with a high-capacity peristaltic pump and filtered through a 35-μm-mesh net. All zooplankton samples were preserved with a 4% formaldehyde solution containing 60 g sucrose·L^{-1} (final formaldehyde concentration ~2%). Macro- and microzooplankton were sampled at every visit to the Kingston station. For longitudinal transects, only macrozooplankton were sampled except in 1989 when both groups were sampled.

The 150-μm-mesh net used in 1987 underestimated the abundance of the small cladoceran *Bosmina longirostris*. We compared numbers of *Bosmina* collected with 150-μm-mesh netting with numbers collected by 35-μm-mesh netting on 12 occasions. *Bosmina* was on average twofold more abundant in the 35-μm samples. We reduced the mesh size for our macrozooplankton samples in subsequent years to provide more accurate estimates of the abundance and biomass of this organism.

In addition to sampling zooplankton, surface water samples were collected for chlorophyll and suspended matter dry weight using a peristaltic pump. Chlorophyll *a* (hereafter chlorophyll) was measured fluorometrically with corrections for pheopigments (Findlay et al. 1991b). Seston samples were filtered onto precombusted, weighed glass fiber filters, dried overnight at 70°C, and reweighed to estimate total suspended matter (Findlay et al. 1991a). River discharge was obtained from a measuring station maintained by the United States Geological Survey (USGS) at Green Island in Troy, NY, just above the tidal portion of the estuary (north of Albany in Fig. 1). We calculated discharge for each of our sampling periods as the cumulative discharge measured by the USGS over the previous 5 d.

To determine zooplankton abundance, either the whole sample or at least 400 individuals were counted in subsamples of each replicate. Macrozooplankton (postnaupliar copepods and cladocerans) were counted with a stereomicroscope at 25× magnification, and microzooplankton (nauplii and rotifers) were counted with an inverted microscope at 100× magnification.

Biomass was estimated from length – dry weight regressions (Bottrell et al. 1976) following the protocol of Bird and Prairie (1985). For each sampling date, lengths were determined for 25–100 individuals of the dominant crustacean taxa and nauplii. Mean dry weights were calculated from length, and biomass was estimated from mean dry weight and abundance data. The volume of selected rotifers was estimated using the formulae of Ruttner-Kolisko (1977). Biomass was calculated by converting volume to wet weight assuming a specific density of 1 which is a slight underestimate based on direct measurements which range from 1.02 to 1.12 (Saunders-Davies and Pontin 1987; Stemberger 1988). Wet weights were converted to dry weights using taxon-specific percentages as determined by Pauli (1989).

Two sampling series were conducted on separate dates in 1987 at the Kingston station to assess variability of zooplankton associated with tidal currents. For both series, samples were taken from an anchored boat over half of a tidal cycle. Triplicate surface water samples were taken every hour for zooplankton, and current speed at 1 m was measured every 30 min with an electronic current meter.

We reviewed the literature to locate studies of riverine and estuarine zooplankton communities where biomass had been estimated. First, we conducted a computerized search and reviewed all papers located in the search and all pertinent references in the bibliographies of those papers. We also systematically reviewed recent publications for information on zooplankton biomass in rivers and estuaries including the *Canadian Journal of Fisheries and Aquatic Sciences* (1987–90), *Estuaries* (1988–90), *Freshwater Biology* (1986–90), *Hydrobiologia* (1988–90), and the *Journal of Plankton Research* (1986–90). Studies were included that provided an estimate of zooplankton biomass for at least monthly samples over either the entire year or, in the case of temperate systems, a significant portion of the year. Studies were excluded when sampling methods were unclear, when only large-mesh netting was used (>200 μm), or when biomass was calculated from volume displacement as opposed to carbon or dry weight estimates.

Results

Variability in Abundance at Sampling and Tidal Scales

Coefficients of variation (CV = standard deviation ÷ mean) for 567 replicate samples of abundance had a median of 0.32 (25th–75th percentiles = 0.19–0.51). Variance of replicate samples was predictable from the mean, and the variability associated with sampling for Hudson River zooplankton was similar to that found in other studies (Pace et al. 1991).

By sampling from an anchored boat on two occasions, we tested whether the water flowing past a station during a tidal

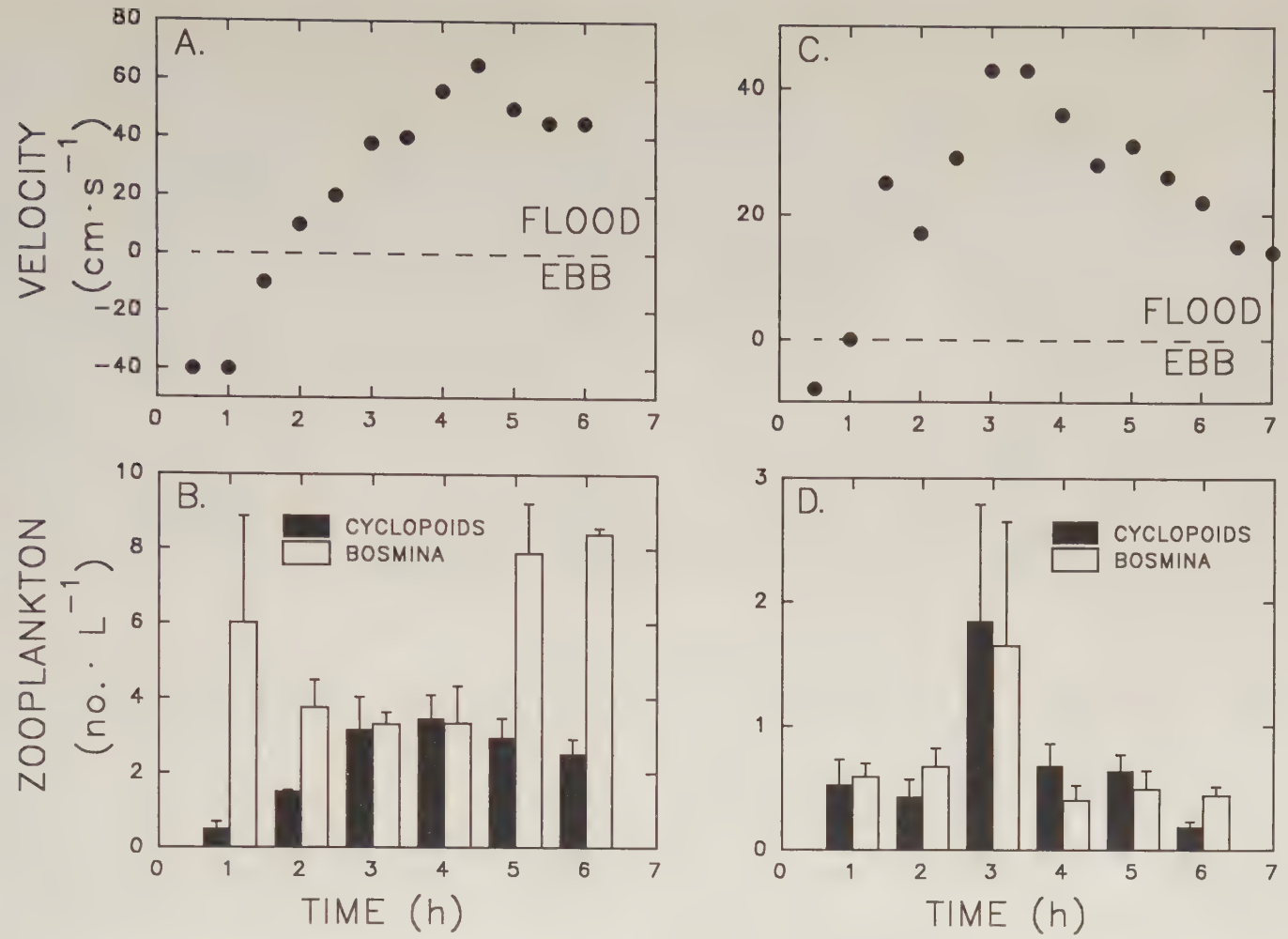

FIG. 2. Variation in current velocity and zooplankton (mean ± SD, $n = 3$) over a portion of a mostly flooding tidal cycle at the Kingston station in (A and B) June and (C and D) October.

excursion was homogeneous with respect to zooplankton. During the first tidal sampling series, samples were taken from 12:30 to 18:00 beginning during ebb tide and ending after peak flood tide (Fig. 2A). There were significant differences in the abundance of the two most common taxa, cyclopoid copepods and *B. longirostris*, during the sampled portion of the tidal cycle (ANOVA: both $P < 0.001$). Cyclopoids were initially present at low densities, and abundances increased during the tidal flood (Fig. 2B). Mean densities of *Bosmina* were similar over the first 4 h and increased after peak flood tide (Fig. 2B). During the second tidal sampling series, samples were taken from 10:15 to 16:15 beginning on the ebb tide near slack low water and ending shortly before slack high water (Fig. 2C). As in the first series, cyclopoid copepods and *Bosmina* were the most abundant taxa. Again, there was heterogeneity across the tide (ANOVA: cyclopoids, $P = 0.02$; *Bosmina*, $P = 0.09$), although variability related to tidal currents was lower than in the first sampling series. For both groups, abundances were similar except near peak flood when a patch of higher densities was sampled (Fig. 2D). We deployed a drogue at the beginning of the tidal flood to estimate the spatial scale of our sampling during the series. The drogue moved approximately 10 km upriver.

Sampling and spatial variation associated with tidal currents indicate that estimates of zooplankton abundance may vary by a factor of two- to fourfold at these scales. In our presentation of results from seasonal and large-scale spatial studies below, we focus on variability that is 10-fold or greater.

Seasonal Dynamics of Hudson Zooplankton

Over the seasonal cycle in the tidal freshwater Hudson, the macrozooplankton fauna is dominated by two crustaceans, the cyclopoid copepod *Diacyclops bicuspidatus thomasi* and the cladoceran *B. longirostris*. The principal rotifers are species of *Keratella, Polyarthra,* and *Trichocerca*. Other species of cladocerans, copepods, and rotifers are present, but the five taxa noted above account for most of the biomass throughout the year.

Zooplankton undergo periods of rapid increase and subsequent rapid decline in summer (Fig. 3). For example, the *B. longirostris* population increased dramatically from 0.01 to 0.1 animals·L^{-1} during the spring flood to >100 animals·L^{-1} in early June. The timing and magnitude of this population bloom were repeated each year, although a lower maximum density was observed in 1989 (Fig. 3A). Net population growth rates (per capita) over the time interval of maximum increase were 0.22–0.37·d^{-1} in each year. Other taxa undergo similar pulses. Postnaupliar cyclopoid copepods are typically present at densities of 1–5 animals·L^{-1}, but occasionally increase to

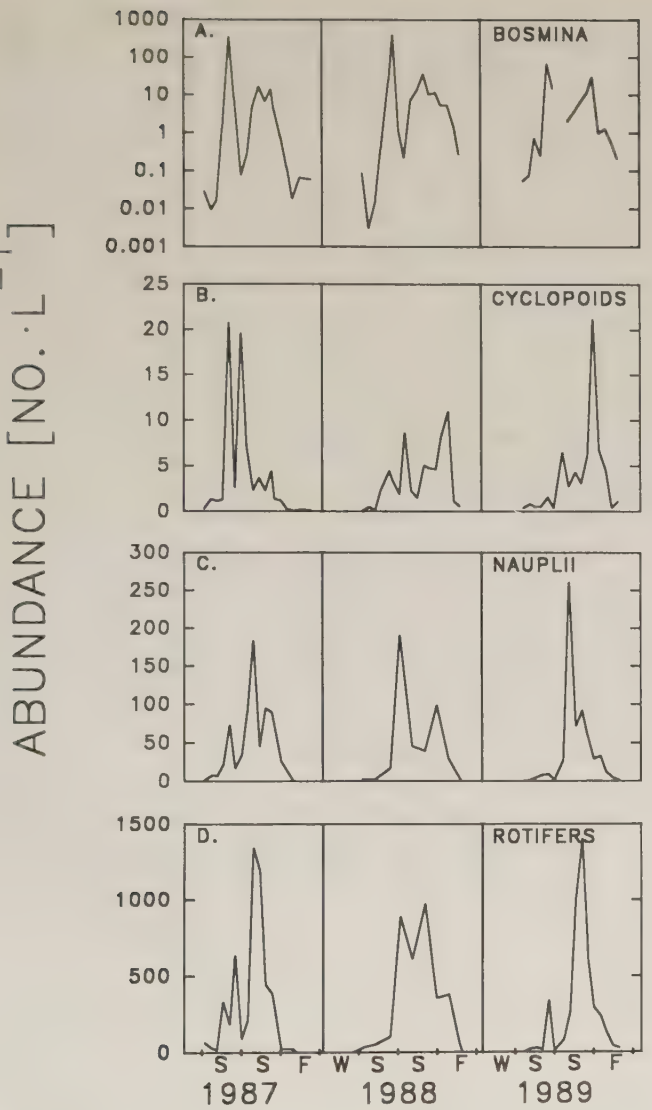

FIG. 3. Seasonal patterns of abundance of (A) *Bosmina*, (B) cyclopoid copepods, (C) nauplii, (D) rotifers at the Kingston station, 1987–89. Each year is divided into 90-d intervals representing winter (W), spring (S), summer (S), and fall (F).

>20 animals·L^{-1} (Fig. 3B). Nauplii oscillate within the range 1–100 animals·L^{-1} except for a single period of peak abundance (>100 animals·L^{-1}) in each summer (Fig. 3C). Similarly, rotifer blooms on the order of 1000 animals·L^{-1} dominated by *Keratella* spp. were observed each summer (Fig. 3D).

The relative biomass of the major groups of zooplankton changes seasonally (Fig. 4). Initially, most of the zooplankton biomass is accounted for by *Bosmina*. After the *Bosmina* population declines in midsummer, cyclopoids and nauplii account for nearly all the biomass. Rotifers are most significant in August. During the fall, biomass is more evenly distributed among the four major groups.

Over the annual cycle, zooplankton biomass at the Kingston station was most strongly correlated with seasonal changes in temperature (Table 1). Chlorophyll was similarly correlated with temperature ($r = 0.65$, $P < 0.0001$). There was also a significant negative relationship between discharge and temperature ($r = -0.70$, $P < 0.0001$). These correlations are consistent with the explanation that a combination of low temperatures and high river flow during much of the year (November to May) limits biological activity in the Hudson. During this period, zooplankton biomass is low (<10 μg dry wt·L^{-1}), while mean monthly discharges are high (>350 $m^3 \cdot s^{-1}$) and temperature is relatively low (<15°C).

To separate temperature and flow effects, the periods from approximately late May to the beginning of October were examined. River temperature exceeded 15°C over this time interval in each year. During this period, zooplankton biomass is high (range 2.0–280 μg dry wt·L^{-1}), and mean monthly discharges are low (<300 $m^3 \cdot s^{-1}$ for June through October). Considering this period over the 3-yr record, zooplankton biomass was not correlated with temperature (range 15–28°C), chlorophyll (range 2.7–67 μg·L^{-1}), or seston (range 6.1–32 mg dry wt·L^{-1}) (Table 1). Furthermore, there was no correlation between zooplankton biomass and chlorophyll from the previous sampling (2 wk earlier), suggesting that time lags in the response of zooplankton to chlorophyll were not important. There was, however, a significant negative relationship between discharge and biomass (Table 1). This relationship is evidence that advection of zooplankton during the summer is a factor regulating community biomass.

Spatial Variation of Hudson Zooplankton

There was substantial spatial variation in zooplankton among stations. The two most abundant taxa of macrozooplankton, cyclopoids and *Bosmina*, were not equally abundant across stations (ANOVA log-transformed data: cyclopoids, $P < 0.0001$; *Bosmina*, $P = 0.005$). Both cyclopoids and *Bosmina* were least abundant (<1 animal·L^{-1}) at the most northerly and most southerly stations (Fig. 5). Between the Hudson and Fort Montgomery stations, cyclopoids were typically present at densities of 2–3 animals·L^{-1} (Fig. 5A). *Bosmina* was similarly abundant across a broad range of the river, but these animals were especially abundant at the Hudson and Kingston stations (Fig. 5B).

We tested whether spatial variation was related to food resources by comparing average chlorophyll concentrations with averages of zooplankton abundance across all surveys. Using average abundance values for each station removes any effect of seasonally important factors such as temperature and discharge. Patterns of spatial variation for *Bosmina* were strongly related to differences among stations in chlorophyll if the most seaward station (Haverstraw Bay) was not considered (Fig. 6). In the case of Haverstraw, the abundance of *Bosmina* was low relative to the chlorophyll concentration. This section of the river is probably near the seaward limit of *Bosmina*, a freshwater species. Densities of cyclopoid copepods were not significantly related to differences in chlorophyll among stations.

The spatial pattern of the *Bosmina* population indicates that there are source areas of the river where net population growth occurs, and similarly, some regions of the river are population sinks (sensu Pulliam 1988). In Fig. 7 the abundance of *Bosmina* at each of our spatial stations is plotted for several of the longitudinal surveys in 1988. The most upstream station, Castleton, is at river km 228, while the most downstream station, Haverstraw Bay, is 153 km south of Castleton (Fig. 1). If there was no net growth or mortality of the *Bosmina* population over this reach, there sould be no change in abundance downstream. When abundance increases between two stations, net population growth is occurring. Similarly, declines between adjacent stations mean that mortality exceeds reproduction. This

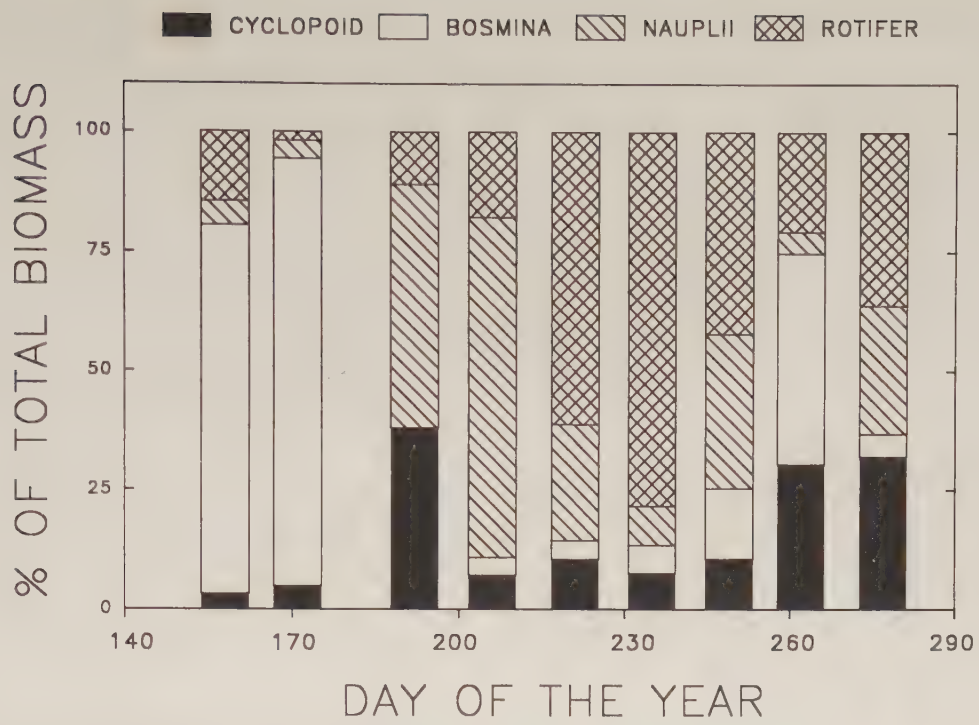

FIG. 4. Percent composition of zooplankton biomass at the Kingston station during 1989 for samples from early June to early October when total biomass exceeded 9 μg dry wt·L^{-1}.

TABLE 1. Correlations at the Kingston station between zooplankton biomass and temperature, river flow, surface chlorophyll, and surface seston concentrations when temperatures ranged from 2.3 to 27.8°C and when temperatures exceeded 15°C. All variables were log transformed prior to analysis. Values are Pearson correlation coefficient (r), number of samples (n), and the probability (P) that the correlation is zero.

	All temperatures			Temperature >15°C		
	r	*N*	*P*	*r*	*N*	*P*
Temperature	0.81	51	0.0001	0.25	32	0.17
Flow	−0.70	47	0.0001	−0.48	31	0.007
Chlorophyll	0.54	51	0.0001	0.06	32	0.74
Seston	−0.17	51	0.24	−0.07	32	0.72

assumes that there are no mechanisms such as vertical migration which might enhance retention. Given the highly mixed water column of the Hudson, vertical migration would only be effective if animals moved to the sediment–water boundary.

Abundances in Fig. 7 have been corrected for the dilution of *Bosmina* by inputs of tributary water. Corrections were made by dividing abundances at each station by the average proportional increase in water from tributary input over the reach. For example, between the Castleton and Hudson stations, cumulative discharge increases about 5% over the Green Island discharge (see study site description) due primarily to freshwater input from Kinderhook Creek (Giese and Barr 1967). Animal densities at Hudson were, therefore, divided by 1.05 to account for the dilution over this interval. We assumed that there were no inputs of *Bosmina* with tributary water, as these streams are small, have high current velocities, and are unlikely to support populations of zooplankton.

When the *Bosmina* population was abundant (Fig. 7A), net population growth occurred over an upriver reach between Castleton and Hudson (June) or Castleton and Kingston (August). Downstream the population declined. In July and September (Fig. 7B), lower abundances of *Bosmina* were observed. There were no areas of net population growth in July. In September, apparent population growth was observed in two sections, Castleton to Hudson and Kingston to Poughkeepsie, but accumulations of animals over these intervals were modest compared with the growth observed in June and August (Fig. 7).

Discussion

Comparison among Systems

To test the hypothesis that advection is an important determinant of zoopankton community biomass, we derived estimates of mean zooplankton biomass from published studies. These studies employed similar methods and sampled zooplankton over at least half of the annual cycle (Table 2). The summarized studies include data from unproductive lakes so that systems where zooplankton are limited by resources are included. We did not review literature for open-water marine systems because these systems are usually not well defined spatially so that water residence time is more difficult to infer.

Patterns of zooplankton biomass conform to the hypothesis that differences in community biomass among systems are partially determined by advection (Fig. 8). Zooplankton biomass is lowest in nontidal rivers and substantially higher in the tidal freshwater Hudson relative to nontidal rivers. The range of annual average biomass observed over 3 yr in the Hudson is lower than the biomass range for saline estuaries. Lakes have the highest zooplankton biomass even though several of these lakes were oligotrophic with low concentrations of nutrients and chlorophyll (Pace 1986). Zooplankton biomass in oligotrophic lakes overlaps, with the highest biomass observed in estuaries.

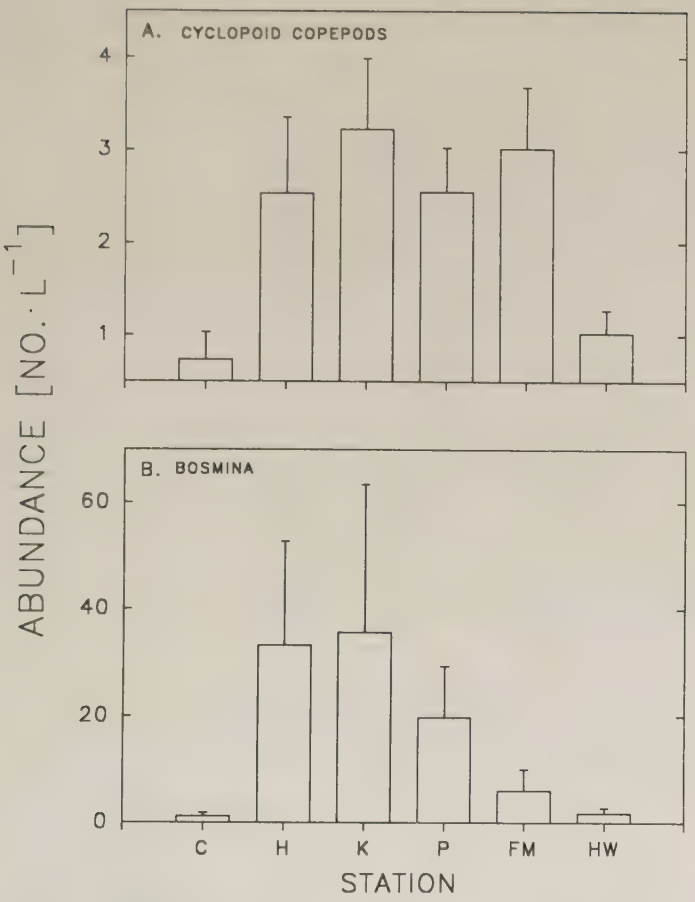

FIG. 5. Spatial variation of (A) cyclopoid copepods (mean ± 1 SE of all surveys) and (B) *Bosmina* (mean ± 1 SE of all surveys). Stations are Castleton (C), Hudson (H), Kingston (K), Poughkeepsie (P), Fort Montgomery (FM), and Haverstraw Bay (HW). See Fig. 1 for locations.

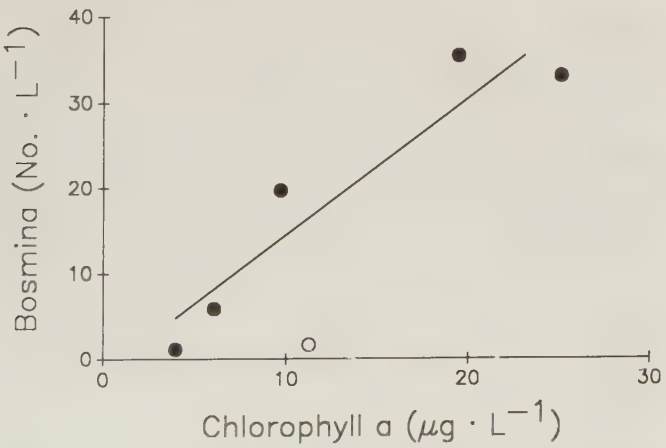

FIG. 6. Station averages of chlorophyll and *B. longirostris* for the longitudinal transects. The line describes a linear relationship excluding the most downstream station (open circle).

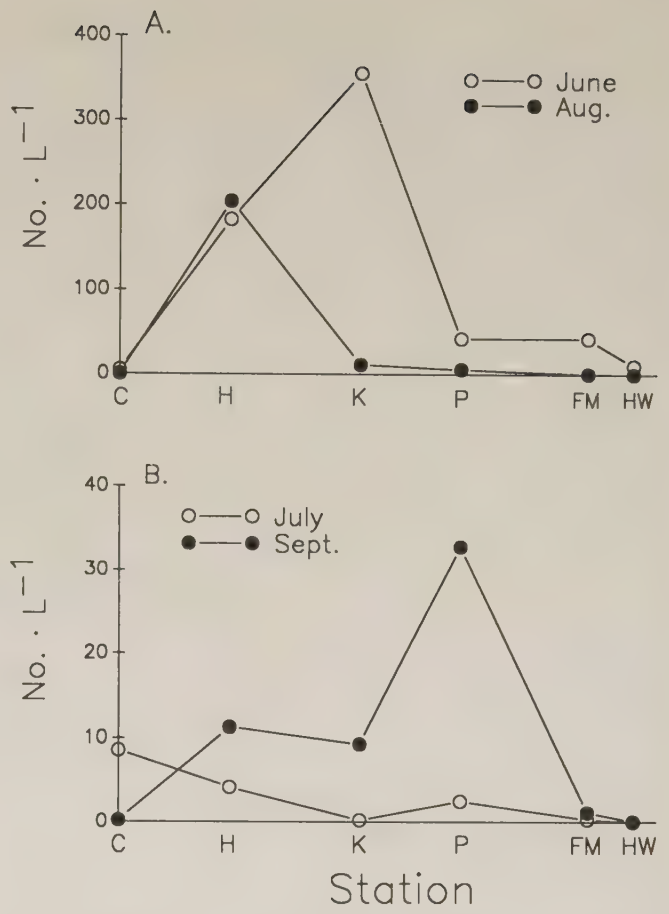

FIG. 7. Abundance of *Bosmina* corrected by dilution from tributary inputs at each station over the 160-km spatial transects in June–September 1988. Changes in abundance between stations reflect net population growth or decline over the river reach. Periods of (A) high net population growth (June and August) and (B) low to negative net population growth (July and September) are illustrated. Stations are Castleton (C), Hudson (H), Kingston (K), Poughkeepsie (P), Fort Montgomery (FM), and Haverstraw Bay (HW). Stations are ordered from north (upstream) to south (downstream). See Fig. 1 for locations.

It has been previously documented for lakes that zooplankton biomass varies positively as a function of algal biomass (McCauley and Kalff 1981). We used chlorophyll in our analysis (when available) to check if potential food resources vary substantially across the environments and thereby confound our test. This was not the case. Average system chlorophyll concentrations overlapped broadly among lakes (range 1.2–29 $\mu g \cdot L^{-1}$), estuaries (range 4.5–17 $\mu g \cdot L^{-1}$), and the Hudson River (range 12–21 $\mu g \cdot L^{-1}$). Differences in zooplankton biomass were not related to chlorophyll across these three system types if we exclude the Orinoco River (regression: $P = 0.69$, $n = 20$). Chlorophyll and zooplankton biomass were very low in the Orinoco (lowest point for rivers in Fig. 8). If this system is included, a regression between chlorophyll and biomass is significant ($P = 0.04$) but accounts for little of the variation ($R^2 = 0.21$) and is entirely due to the low values observed in the Orinoco River. Low resources in addition to strong advection may be important in regulating zooplankton biomass in this river. In contrast with the Orinoco, other rivers such as the Danube have high chlorophyll concentrations (56 $\mu g \cdot L^{-1}$) but relatively low zooplankton biomass (6 μg dry wt $\cdot L^{-1}$).

Advection Hypothesis at the Population and Community Level

Both within the Hudson River and among different types of aquatic systems, advection appears to be important in regulating zooplankton community biomass. The relative time scales of water turnover and population processes will determine if

TABLE 2. Summary of studies used in comparative analysis of zooplankton biomass. Mesh refers to the size (μm) of the finest net used to filter water.

Study	Systems	Gear	Mesh	Period	Reference
Apure River	1	Pump	35	15 mo	Saunders and Lewis 1988
Orinoco River	1	Pump	35	Annual, 3 yr	Saunders and Lewis 1989
Danube River[a]	1	Pump	75	Apr.–Sept.	Bothár and Kiss 1990
Freshwater Hudson	1	Pump	35	Apr.–Oct., 3 yr	This study
North inlet, estuary	1	Net	158	20 mo	Lonsdale and Coull 1977
Naragansett Bay[b]	2	Pump	60	Mar.–Oct.	Durbin and Durbin 1981
San Francisco Bay	3	Pump	64	Annual	Ambler et al. 1985
Quebec lakes	12	Pump	35	May–Sept.	Pace 1986

[a]Data for crustacean zooplankton only.
[b]Data for *Acartia* spp. (Copepoda) only.

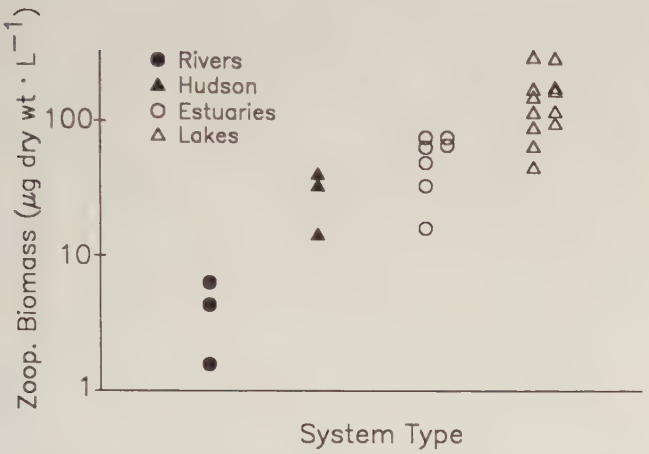

FIG. 8. Average zooplankton biomass versus type of system. Data categories are rivers, annual averages (1987–89) of the tidal freshwater Hudson, saline estuaries, and lakes. Some estuarine and lake values are offset for clarity.

advection is important in any given circumstance (Ketchum 1954). Most lakes have water residence times on the scale of years (e.g. data summarized in Rasmussen et al. 1989), while zooplankton generation times vary from days to months (Allan and Goulden 1980). In lakes, advective transport is usually negligible, and zooplankton community biomass is related to other factors (e.g. Pace 1986; Yan 1986). In rivers and estuaries, water residence times are short enough to influence zooplankton dynamics (Ketchum 1954; Miller 1983). Advective transport in these systems may move animals away from locations favoring net population growth into less favorable locations. These less favorable locations may be sites with fewer resources, more predators, or higher salinity.

The movement of animals from areas favorable for population growth is evident for the Hudson River population of *B. longirostris*. Spatial heterogeneity within the river is high for this population. The region of the river from south of the Castleton station to Kingston (Fig. 1) is the area in which net population growth generally occurs (Fig. 7). A possible explanation for this pattern is that chlorophyll concentrations are also higher over this stretch of the river so that resources are, during some periods of the year, sufficient to support reproduction at rates that exceed mortality and downstream losses. For the population downstream of the Kingston station, reproduction generally cannot compensate for mortality and advective losses, and the population declines.

At the community level, our comparative analysis of zooplankton biomass provides evidence for the importance of advection. The analysis is limited in two ways. First, we do not have direct estimates of water residence time. System type (lake, river, estuary) serves as an imperfect analogue of relative water residence time. A second limitation is the small number of rivers and estuaries for which we were able to obtain data on zooplankton biomass. We conducted an extensive computerized search of the literature as well as a systematic review of a variety of aquatic journals (total = 60 volumes) to find appropriate data for zooplankton biomass in rivers and estuaries. We conclude that our comparison strongly suggests that advective transport may limit zooplankton biomass in flowing water environments; however, more systems and direct estimates of water residence times are needed to adequately test the hypothesis.

Our results suggest that hydrological features such as water residence time may be useful in comparing communities across environments. Water residence time was an important property distinguishing differences in the abundance of phytoplankton among 641 rivers, reservoirs, and lakes surveyed by government agencies in the United States (Søballe and Kimmel 1987). In this study, water residence time was calculated from stream input and water volume for lakes and reservoirs or approximated from drainage area and average annual flow for rivers. Average residence times were 18, 528, and 1073 d for rivers, reservoirs, and lakes, respectively. Systems with short water residence times had a lower abundance of phytoplankton as determined by cell counts. Rivers had lower phytoplankton relative to reservoirs and lakes even though phosphorus, a nutrient often in limiting supply, was typically higher in rivers (Søballe and Kimmel 1987). These results for phytoplankton are very similar to the inferences we have made for zooplankton. The significance of advection should be more important for zooplankton than phytoplankton, since zooplankton have longer generation times and cannot compensate as quickly for advective losses. A simple model of fjord plankton by Aksnes et al. (1989) supports this idea. Advection was more important than growth in determining community biomass, especially for zooplankton residing in surface waters. For phytoplankton, growth within the fjord dominated over losses due to transport.

In this paper, we have focused on the significance of advective transport for zooplankton communities. There are other hydrological phenomena such as turbulence that may also be important. In a system like the Hudson, tidal currents and winds produce turbulence which constantly mixes the water column. It is not known if turbulence of the degree found in the Hudson is an important factor limiting community biomass. Theoretical

analyses suggest that turbulence may lead to either increased or decreased predator–prey contacts (Rothschild and Osborn 1988; Evans 1989; Davis et al. 1991). Experimental studies of the feeding and behavior of copepods under turbulent and nonturbulent conditions indicate that there are both positive and negative effects of turbulence on these animals (Costello et al. 1990; Marrasé et al. 1990). The potential consequences of turbulence for particular species may or may not translate into differences in community biomass. Turbulence must be higher on average in river and estuarine environments relative to lakes. This may be an additional important feature distinguishing zooplankton community biomass between these environments, but the net impact of turbulence on zooplankton is not known (Marrasé et al. 1990; Davis et al. 1991).

Riverine Zooplankton Communities

The paucity of data on zooplankton biomass in rivers reflects the small amount of research that has been done on these communities. There are few generalizations about either the structure or regulation of these communities other than the significance of transport (Hynes 1970; Saunders and Lewis 1989). Community structure, however, appears very similar in the rivers which have been studied. Bosminids and cyclopoid copepods are often two of the dominant taxa either in terms of biomass or abundance, as observed, for example, in the lower Murray (Shiel et al. 1982), Orinoco (Saunders and Lewis 1989), Danube (Bothár and Kiss 1990), St. Lawrence (Bousfield et al. 1975), and the Hudson (present study). In addition, zooplankton community size structure appears to be more uniformly dominated by small species than is the case in lakes (Shiel et al. 1982). A number of factors probably favor small zooplankton in rivers. Small species have short generation times which could reduce the impact of advective losses. Small species feed and grow more successfully than large species when high concentrations of filamentous and/or toxic algae are present (Webster and Peters 1978; Fulton and Pearl 1987; Fulton 1988; Gilbert 1990), as is often the case in rivers (Hynes 1970) including the Hudson, where high densities of filamentous and gelatinous blue-greens occur (Marshall 1988). Finally, small species may be favored in turbid environments. Experimental studies of rotifers indicate that these species are less susceptible than cladocerans to the negative effects of high concentrations of suspended clays (Kirk and Gilbert 1990).

Only recently have aquatic ecologists begun a more formal appraisal of similarities and differences among aquatic systems (e.g. Nixon 1988), and many of these evaluations have focused on comparisons of freshwater and marine environments where chemical contrasts are important (e.g. Caraco et al. 1989). There has been relatively little work comparing community or ecosystem properties across rivers, reservoirs, lakes, estuaries, and oceans. Our results and those of Søballe and Kimmel (1987) indicate that differences in physical environment are likely to be central in determining patterns at this scale.

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Pharmacokinetics, Bioavailability, Distribution, and Metabolism of Sulfadimethoxine in the Rainbow Trout (*Oncorhynchus mykiss*)

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Kleinow, K. M., W. L. Beilfuss, H. H. Jarboe, B. F. Droy, and J. J. Lech. 1992. Pharmacokinetics, bioavailability, distribution, and metabolism of sulfadimethoxine in the rainbow trout (*Oncorhynchus mykiss*). Can. J. Fish. Aquat. Sci. 49: 1070–1077.

Sulfadimethoxine (SDM), a sulfonamide antibacterial drug, was examined in regards to bioavailability, pharmacokinetics, distribution, plasma protein binding, mass balance, and metabolism in the rainbow trout (*Oncorhynchus mykiss*). Analysis of SDM pharmacokinetics when determined by ^{35}S counting (parent/metabolite combination) and HPLC (parent) provided estimates for $t_{1/2}\alpha$, $t_{1/2}\beta$, V_{ss}, and Cl_b, of 0.63/0.38 (h), 17.0/15.9 (h), 500.8/421.6 (mL/kg), and 22.7/21.8 $mL \cdot kg^{-1} \cdot h^{-1}$, respectively. Multiple dose administration of [^{35}S]SDM resulted in a terminal $t_{1/2}$ of 35.2 h. Sodium SDM (42 and 126 mg/kg) and free drug (42 mg/kg) oral bioavailabilities were 63, 50, and 34%, respectively. Plasma protein binding (15.8 ± 5.1%) was nonsaturable and nonspecific. Tissues attained the highest levels of SDM equivalents in the bile followed by the intestine, liver, blood, skin, kidney, spleen, gill, muscle, and fat, respectively. Mass balance studies demonstrated a minor role for branchial ((0.6 ± 0.24%) 25 h) and urinary ((5.46 ± 2.24%) 24 h) routes of elimination over the first 24 h. SDM and N_4-acetylated SDM (N_4-A-SDM) were the major constituents for branchial and urinary routes, respectively. Metabolite analysis for select tissues demonstrated a predominance of N_4-A-SDM in bile, SDM in plasma, and N_4-A-SDM in the liver 20 h after dosing.

La sulfadiméthoxine (SDM) a fait l'objet d'un examen portant sur la biodisponibilité, la pharmacocinétique, la répartition, la liaison aux protéines plasmatiques, le bilan massique et le métabolisme chez la truite arc-en-ciel (*Oncorhynchus mykiss*). L'analyse de la pharmacocinétique de la SDM par comptage du ^{35}S (produit de départ-métabolite) et CLHP (produit de départ) a permis d'obtenir des valeurs de $t_{1/2}\alpha$, $t_{1/2}\beta$, V_{ss} et Cl_b s'élevant à, respectivement, 0,63/0,38 (h), 17,0/15,9 (h), 500,8/421,6 (mL/kg) et 22,7/21,8 $mL \cdot kg^{-1} \cdot h^{-1}$). L'administration de doses multiples de [^{35}S]SDM a donné une valeur $t_{1/2}$ définitive de 35,2 h. Les biodisponibilités buccales de la SDM-sodium (42 et 126 mg/kg) et du SDM libre a (42 mg/kg) étaient respectivement de 63, 50, et 34 %. La liaison aux protéines plasmatiques non saturable et non spécifique était de (15,8 ± 5,1 %). Les teneurs les plus élevées de SDM équivalents ont été notées, par ordre décroissant, dans la bile, l'intestin, le foie, le sang, la peau, le rein, la rate, les branchies, les muscles et la graisse. Les études du bilan massique ont montré que l'élimination par les branchies ((0,6 ± 0,24 %) 25 h) et l'appareil urinaire ((5,46 ± 2,24 %) 24 h) était peu importante au cours des 24 premières heures. La SDM et la SDM N_4 acétylée (SDM-N_4-A) constituaient les principaux produits éliminés par les branchies et l'appareil urinaire. L'analyse des métabolites de certains tissus a montré la prédominance du SDM-N_4-A dans la bile, de la SDM dans le plasma et du SDM-N_4-A dans le foie, 20 h après l'administration.

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Sulfonamides have been widely used in food-producing animals for the treatment of bacterial and coccidial diseases (Bushby 1980; Jenkins 1987; Marusich et al. 1969; Rehm and White 1970). Although this large group of antimicrobial agents shares a common mechanism involving inhibition of folate synthesis, these compounds demonstrate by nature of their varying structures a wide diversity in their bioavailability, metabolic, and pharmacokinetic characteristics (Jenkins 1987; Struller 1968). In addition to structure-related differences, significant variations in pharmacokinetic behavior have also been noted for individual sulfonamides when examined in different animal species.

Sulfadimethoxine (SDM), generally regarded as a long-acting sulfonamide in mammals, has been shown to be effi-

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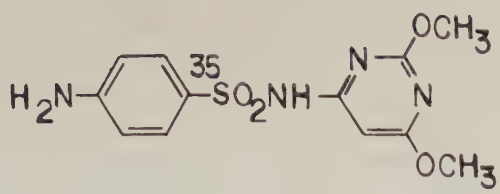

FIG. 1. Structure of SDM.

cacious in the treatment of bacterial diseases in cultured fish. Most notably, SDM has demonstrated activity alone and in combination with the potentiator ormetoprim for control of furunculosis in salmonids (Bullock et al. 1974) and enteric septicemia in ictalurid fishes (Bullock and Herman 1985). While efficacy considerations in support of drug approval in catfish and trout have been well investigated, pharmacokinetic and metabolic issues have been addressed only recently in the channel catfish (*Ictalurus punctatus*) (Squibb et al. 1988). Similar data as collected for the catfish have yet to be reported in the literature for a coldwater fish species of aquacultural importance. The objectives of the present study were to provide pharmacokinetic, bioavailability, metabolic, and distribution data for characterization of SDM in the rainbow trout (*Oncorhynchus mykiss*).

Materials and Methods

Animals

Rainbow trout obtained from either Hideaway Springs Trout Farm, West Bend, WI (180–650 g), or Greers Ferry National Fish Hatchery, Heber Springs, AR (650–1094 g), were used in the experiments. The fish were maintained under flow-through conditions in dechlorinated tap water at 13 ± 1.0°C on a 12 h dark/12 h light photoperiod for at least 2 wk prior to the experiments. The fish were fed a maintenance diet of Silvercup® trout pellets (Murray Elevators, Murray, UT) on a daily basis up to the day prior to surgical preparation or experimentation.

Materials

SDM as free drug or sodium salt was used for experimentation. The stable compounds were obtained from Sigma chemical company. SDM radiolabelled with ^{35}S (Fig. 1) was obtained as the free drug from Amersham International and then purified over silica gel to a radiochemical purity of greater than 97%. The sodium salt of the radioisotopic base was formed by the pH-adjusted addition of sodium bicarbonate. Following specific activity determination, the corresponding combination of radioisotope and stable compound was administered either in the food or as an intravenous dose as described for individual experiments. The food was prepared to a gel-like consistency as a slurry of water and maintenance diet. Intravenous doses of the compound were administered in a phosphate-buffered Ringers solution (pH 7.4) (Erickson 1984) as the carrier vehicle. All chemicals and solvents used were of the highest quality commercially available.

Sampling and Experimental Procedures

The fish used for pharmacokinetic, bioavailability, metabolism and protein binding studies were individually housed during the experiments in flow-through chambers that facilitated the use of aortic cannulas, indwelling stomach tubes, and urinary catheters (Kleinow 1991). This system permitted the administration of SDM intravenously or by gavage as well as access for sequential blood and urine sampling in free-swimming trout. The complement of hardware used was dependent upon sample requirements for individual experiments. For the catheter, cannula, and stomach tube implants, tricaine methanesulfonate (Argent Labs, Redmond, WA) was used for induction and maintenance of anesthesia at 100 and 50 mg/L, respectively. The fish were allowed at least 18 h following surgical manipulation and before experimentation for clearance of the anesthetic and reestablishment of normal renal function (Hunn and Willford 1970) and cytochrome P-450 activity (Fabacher 1982; Kleinow et al. 1986). The dorsal aorta cannulation and urinary catheterization were performed for appropriate experiments as a modification of the procedure described by Beyenbach and Kirschner (1975). In part, the modification included attachment of the cannula and catheter to the dorsal fin to provide a central pivot for a complete range of movement in the free-swimming fish. The indwelling stomach tube consisted of an elbow secured through the snout with the placement of a latex tube externally and internally by a rigid tube extending from the elbow through the esophagus ending just cranial to the body of the stomach.

Radioactivity Measurements

Radioisotope content was determined in appropriate samples using a Packard Tri-Carb liquid scintillation counter (Packard Instrument Co., Downers Grove, IL). Tissue samples (80–140 mg) were digested in 0.5 mL of TS-1 (Res. Prod. International Corp., Mt. Prospect, IL) tissue solubilizer at 50°C for 24 h. The samples were cooled and neutralized with 36 μL of glacial acetic acid before addition of scintillant (Ultima Gold-Packard, Downers Grove, IL). Blood samples were centrifuged at 12 000 rpm (13 000 *g*) for 5 min. Plasma samples (40 μL) were added directly to the scintillant and counted for radioactivity. Sample radioactivity was corrected for quench, counting efficiency, decay, and background.

HPLC Analyses of SDM and Metabolites

Plasma and urine samples (500 μL) collected for HPLC analysis were acidified with an equal amount of 0.01 N HC1, vortexed, and exhaustively extracted with ethyl acetate. Samples were back-extracted and appropriately pooled into aqueous and ethyl acetate extractable fractions. Ethyl acetate fractions were dried by adding anhydrous sodium sulfate. Tissues were homogenized with 0.01 N HC1. The resulting suspension was exhaustively extracted with hexane and ethyl acetate in succession. All phases were concentrated and stored under nitrogen in light-protected vials until analysis.

Liquid chromatographic (HPLC) analyses of SDM and metabolites were accomplished isocratically on a C_{18} reverse-phase column using a 40:60 methanol – 0.01 M acetate buffer, pH 4.0 (M. O. James, pers. comm.), mobile phase. Authentic SDM and N_4-acetylsulfadimethoxine (N_4-A-SDM) standards confirmed by NMR were used as retention standards on a daily basis. Quantification of stable compound was accomplished using a standard curve constructed on the basis of an extracted compound/internal benzanilide standard ratio. Areas under the curve (AUC's) were used for formulation of these comparisons.

Pharmacokinetic Analysis

The data resulting from intravenous dose administration were initially subjected to graphical analysis by a semilogarithmic plot of log plasma concentration (C_p) against time. Linear regression of the ln of the data and the method of residuals (Renwick 1982) were used to define initial estimates of the alpha and beta elimination rate constants as well as the *y*-intercepts (A, B). The data were best described and then fitted to a biexponential model. Corresponding $t_{1/2}$'s were calculated from $t_{1/2} = 0.693/\lambda$ where λ is the slope for the respective alpha and beta phases. All subsequent pharmacokinetic analyses were performed according to statistical moment theory (Gibaldi and Perrier 1982).

Single- and Multiple-Dose Pharmacokinetics

Sodium SDM (42 mg/kg) was administered as a single intravenous dose via aortic cannula to each member of two groups of fish. The first group composed of three fish were administered sodium [^{35}S]SDM. Pharmacokinetic calculations for this group were based upon [^{35}S]SDM equivalents. The second group ($N = 5$) was administered stable sodium SDM and the subsequent kinetics based upon HPLC-derived values of parent compound. This dual protocol was used to examine methodological approaches and the contribution of parent and metabolites to the kinetic profile. Blood samples for both groups (100 μL for [^{35}S]SDM; 500 μL for HPLC analysis) were collected at 10 and 20 min and 1, 4, 8, 12, 20, 24, 48, 72, and 96 h post-dosage. Additional samples were taken at 120 and 144 h for [^{35}S]SDM-administered fish.

Multiple doses of sodium [^{35}S]SDM were administered intravenously to three fish as five individual doses of 42 mg/kg at time 0 and 24, 48, 72, and 96 h following the initial dose. Five dose administrations were selected on the basis of the $t_{1/2}$ of SDM following a single-dose administration (i.e. $3.3 \times t_{1/2}$ = time to apparent steady state). Blood samples were collected during the rise to steady state at 10 min and 24 h following each administration. Animals were allowed to depurate following the 96-h dose. Blood samples were collected during the depuration phase at 10 min and 1, 3, 8, 24, 48, 72, 96, 120, and 144 h following the fifth and final dose.

Oral Bioavailability

A total of 15 fish were dosed with [^{35}S]SDM via gavage for the purpose of examining intestinal bioavailability. The dose for each fish was mixed in a 2-mL slurry of maintenance diet. The dose was followed by a 2-mL dietary chaser to ensure delivery from the stomach tube. SDM as the sodium salt was administered as a single dose to six fish at 42 mg/kg and three fish at 126 mg/kg. The free drug (42 mg/kg) was similarly administered to six fish. Blood samples were collected as previously described at 1, 4, 8, 12, 16, 20, 24, 28, 36, 48, 72, 96, 120, and 144 h post-dosage. The samples were processed as for single-dose pharmacokinetic studies using [^{35}S]SDM equivalents. In another experiment, six fish were administered stable sodium SDM by gavage. Plasma concentrations were assessed as parent compound with HPLC analysis. The bioavailability of oral administrations was determined with the areas under the intravenous and gavage plasma concentration versus time curves as described by

$$\frac{\text{Dose}_{\text{i.v}} \times \text{AUC}_{\text{oral}}}{\text{Dose}_{\text{oral}} \times \text{AUC}_{\text{i v.}}} \times 100.$$

The zero moment (area under the curve, AUC) was estimated by the equation

$$\text{AUC} = {}_0\int^{\infty} C_p dt$$

where C_p was the plasma drug concentration at time t. The AUC from $t = 0$ to time of last measured concentration (t^*) was calculated according to Simpson's rule. The AUC from t^* to infinity was estimated by C_p^*/λ_z, where C_p^* was the concentration at t^* and λ_z was the elimination rate constant calculated as -2.303 multiplied by the slope of the terminal phase of the log plasma concentration versus time curve.

Systemic Bioavailability from Isolated Gut Segments

A total of nine fish was anesthetized as previously described. Surgical isolations of the stomach including the proximal ascending intestine, the proximal descending intestine, and distal descending intestine were performed from midventral incisions in the body wall in separate groups of three fish. The lumen of the gastrointestinal segments was isolated by placing a double set of ligatures around the gut at the distal extent of the area of interest. All ligatures were placed and secured in such a manner such as not to impede major routes of blood flow or to cut the gut wall. Once the distal ligature was situated, the proximal ligature was placed, but not tightened, 3 cm proximal to the distal. A beveled needle was introduced through the gut wall into the lumen, advancing under the proximal ligature into the isolated segment. Stable and [^{35}S]SDM (42 mg/kg) dissolved in teleost Ringers was introduced as a mixture into the segment under consideration for each. The segment was subsequently ligated and the body wall closed with a close apposition suture pattern. The animals were allowed to recover from anesthesia and were placed in holding tanks for 24 h. Subsequent to the holding period, the animals were anesthetized, weighed, bled for samples, and, following examination for preparation integrity, the appropriate gut section harvested with ligatures intact. Gut sections were individually weighed and homogenized. Total volumes of the homogenates were recorded and aliquots prepared for scintillation counting. Systemic bioavailability was evaluated according to the disappearance of SDM from the gut and the rate of appearance in the plasma.

Tissue Distribution

The tissue distribution of sodium SDM and metabolites was determined following administration of sodium [^{35}S]SDM (42 mg/kg) by gavage in food to 44 trout. Four animals were sampled at each of 11 times; 4, 12, 18, and 24 h and 3, 7, 10, 14, 21, 35, and 49 d. Blood, liver, bile, gill, kidney, spleen, intestine, skin, muscle, and fat were collected for total SDM-derived radioactivity determinations. The $t_{1/2}$'s of [^{35}S]SDM in select tissues were calculated using linear regression of post-absorption data. Residue $t_{1/2}$ calculations were performed as previously described.

Plasma Protein Binding

Three fish were dosed with sodium [^{35}S]SDM (42 mg/kg) via preplaced dorsal aortic cannulae. Plasma resulting from blood collected at 2, 6, 24, and 72 h post-dosing was immediately ultrafiltered (13°C) in Amicon Centrifree micropartition units (Amicon Corp., Danvers, MA). [^{35}S]SDM concentration was determined before and after filtration for determination of

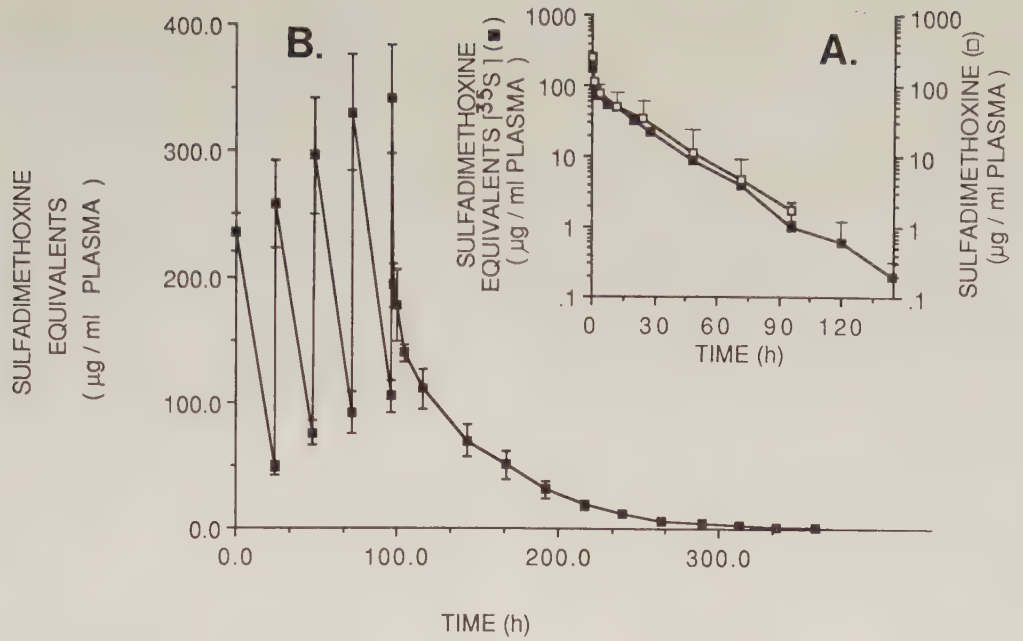

FIG. 2. Semilog plot of (A) total plasma ^{35}S concentration (parent and metabolites) versus time following a single i.v. dose (42 mg/kg) of [^{35}S]SDM to rainbow trout (■) (N = 3) and SDM concentration versus time following a single i.v. dose (42 mg/kg) of stable SDM to rainbow trout (□) (N = 5) and (B) plasma total radioactivity versus time following five i.v. doses (42 mg/kg) of [^{35}S]SDM (steady state) to rainbow trout (N = 3). All points are means ± SD.

protein binding. Nonspecific binding of the drug onto the filtration unit was determined and the appropriate correction employed.

Concentration-dependent plasma protein binding studies were also performed in vitro for trout using pooled plasma from four control animals. Sodium SDM was added to aliquots of plasma from each species through a concentration range of 0.2 to 10 mM. Ultrafiltration procedures were used for protein binding determinations. Subsequent data were examined via Scatchard analysis.

Mass Balance

Two free-swimming fasted fish were used for mass balance studies due to the labor-intensive nature of the studies. Aortic cannulae, urinary catheters, and water collection were used to assess the relative contribution of each route to the elimination of the drug following intravenous administration.

[^{35}S]SDM levels and metabolite profiles of select samples were determined in the plasma, urine, and water during the course of the experiment. Urine was compositely collected at 4-h intervals through the first 24 h and then at 36, 48, 72, and 96 h, post-administration. Radioactivity and urine volume were used for interval and cumulative drug quantification. Plasma was collected at 0.5, 0.8, 2, 4, 8, 12, 24, 36, 48, 72, and 96 h post-administration. XAD-2 resin extraction of water from the first 25 h was used to collect branchial elimination products. Branchial elimination was quantified as total radioactivity of a resin methanol series elution. Where appropriate, samples were subjected to extraction and metabolite identification by HPLC as previously described.

Metabolism Study

Sodium [^{35}S]SDM (42 mg/kg) was administered to three fish by gavage in a slurry of maintenance diet. Bile, plasma, and liver samples were collected 20 h post-administration. SDM and the major metabolite N_4-A-SDM were extracted, isolated by HPLC, and quantified by ^{35}S counting.

Statistics

All statistical comparisons were performed with ANOVA or the t-test at $P \leq 0.05$ (Sokal and Rohlf 1973).

Results and Discussion

The mean plasma [^{35}S]SDM and stable SDM concentrations following a single 42 mg/kg i.v. administration are plotted separately in Fig. 2A. The data demonstrate that drug disappearance from plasma is biexponential. Pharmacokinetic parameters for the single i.v. dose using both means of measurement are listed in Table 1. Pharmacokinetic parameters were not significantly different between [^{35}S]SDM (parent and metabolites) and SDM (parent) derived values. These results suggest that SDM kinetics may be the major determinant of the composite [^{35}S]SDM-generated values. Plasma $t_{1/2}$'s for the α distribution and β elimination phases ($t_{1/2}\alpha$ and $t_{1/2}\beta$) were 0.63/0.38 ([^{35}S]SDM/SDM) and 17.0/15.9 h respectively. In comparison, male American lobster (*Homarus americanus*) exhibited $t_{1/2}\alpha$ = 1.96 h and $t_{1/2}\beta$ = 71.5 h (Barron et al. 1988), while catfish demonstrated corresponding values of 0.06 and 12.8 h (Squibb et al. 1988).

The apparent volume of distribution (V_1) of the central compartment (170.8 mL/kg/148.5 mL/kg ([^{35}S]SDM/SDM)) and (V_{ss}) composite volumes (central and peripheral) (500.8/421.6 (mL/kg)) indicates a distribution beyond the vascular compartment. These values, however, represent a curtailed distribution into the extravascular body water, perhaps as a result of limitation to the extracellular fluid space. The V_{ss} values were greater for trout than those reported for a variety of mammalian

TABLE 1. Pharmacokinetic parameters (means ± SD) in rainbow trout following single i.v. administration (42 mg/kg) of [^{35}S]SDM (N = 3) or SDM (N = 5) and multiple i.v. dosage (5 × 42 mg/kg) of [^{35}S]SDM (N = 3). [^{35}S]SDM measured as total SDM equivalents (radioactivity) and SDM measured as parent SDM by HPLC. $t_{1/2}\alpha$ = biological half-life of ^{35}S or parent SDM in plasma during drug distribution phase; $t_{1/2}\beta$ = biological half-life of ^{35}S or parent SDM in plasma during terminal elimination phase; V_1 = volume of central compartment; V_{SS} = volume of distribution at steady state; Cl_t = intercompartmental clearance; CL_b = whole-body clearance.

Parameter	Single dose[a]		Multiple dose (5 × [^{35}S]SDM)
	[^{35}S]SDM	SDM	
$t_{1/2}\alpha$ (h)	0.63 ± 0.33	0.38 ± 0.06	0.20 ± 0.09
$t_{1/2}\beta$ (h)	17.0 ± 1.5	15.9 ± 5.4	35.2 ± 5.3[b]
V_1 (mL/kg)	170.8 ± 19.4	148.5 ± 6.5	—
V_{SS} (mL/kg)	500.8 ± 41.6	421.6 ± 57.4	—
Cl_t (mL·kg^{-1}·h^{-1})	148.8 ± 115.9	164.0 ± 16.5	—
Cl_b (mL·kg^{-1}·h^{-1})	22.7 ± 1.1	21.8 ± 9.9	—

[a]No significant differences between [^{35}S]SDM and SDM ($P \leq 0.05$).
[b]Significantly different from $t_{1/2}\beta$ of single-dose administration ($P \leq 0.05$).

species but smaller than those reported for the catfish (662 mL/kg) (Squibb et al. 1988) and the lobster (1369 mL/kg) (Barron et al. 1988). Trout total body clearances (Cl_b) of 22.7/21.8 ([^{35}S]SDM/SDM) mL·kg^{-1}·h^{-1} and intercompartmental clearances (Cl_t) of 148.8/164.0 mL·kg^{-1}·h^{-1} indicate that Cl_b appears to control elimination of the drug in contrast with a slow release from peripheral storage depots.

The accumulation and elimination of [^{35}S]SDM following a 42 mg/kg i.v. dose administered once a day for 5 d is shown in Fig. 2B. The upper excursions represent the maximum plasma concentrations as determined 10 min after i.v. dosing, while the minimum concentrations were measured 24 h later just prior to the administration of the subsequent dose. Mean [^{35}S]SDM equivalent concentrations following multiple dosing increased 82 μg/mL above that of a single-dose administration, with a peak mean value of 342 μg/mL. The pharmacokinetic parameters available from data on the fifth dose and final elimination phase following log conversion are listed in Table 1. It should be noted for the multiple-dose study that the $t_{1/2}\alpha$ is a rough estimate due to the limited number of samples collected during the short redistribution phase. This limitation precluded meaningful calculation of V_1, V_{ss}, Cl_t, and Cl_b. Nevertheless, importantly, the $t_{1/2}$'s of the terminal elimination phase $t_{1/2}\beta$ for the single- and multiple-dose administrations were extended from 17.0 to 35.2 h, respectively. Preliminary studies have not substantiated a biotransformational basis to the observed differences. Although confirmatory studies have not been carried out, it appears likely that saturation of an excretory process has been evoked.

The [^{35}S]SDM equivalent verses time plots following single but separate administrations by gavage for the SDM sodium salt at 42 and 126 mg/kg as well as for the free drug at 42 mg/kg are given in Fig. 3A. [^{35}S]SDM administered as either the sodium salt or free drug demonstrated peak absorption between 10 and 24 h, with the specific times dependent upon the chemical form examined. It is of interest to note that catfish demonstrated peak plasma levels 3–6 h post-administration (Squibb et al. 1988). Discrepancies of these findings with those of the current study may be a reflection of differences between the two species, experimental design of administration techniques, anatomical sites of absorption, or temperature-associated rates of peristalsis.

In trout the sodium salt exhibited a significantly greater bioavailability (63 ± 9.2%) compared with that of the free drug

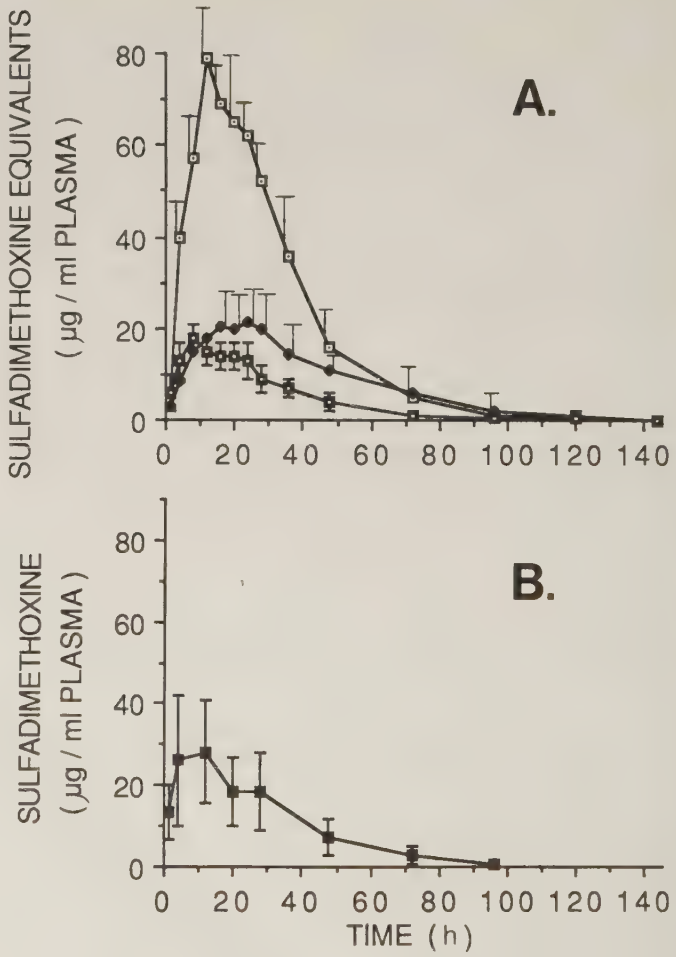

FIG. 3. Plasma concentration of (A) total ^{35}S versus time following oral administration of [^{35}S]SDM to rainbow trout as the sodium salt at 42 mg/kg (◆) (N = 6) and 126 mg/kg (□) (N = 3) and as the free drug at 42 mg/kg (■) (N = 6) and (B) SDM versus time following oral administration of stable SDM to rainbow trout at 42 mg/kg (N = 6).

(34 ± 8.5%) when administered in the same dietary food mixture at 42 mg/kg. Furthermore, the normalized bioavailability of the sodium salt was reduced when the [^{35}S]SDM dose was increased from 42 to 126 mg/kg even though absolute peak

TABLE 2. Systemic bioavailability and resultant plasma concentrations (means ± SD) of [^{35}S]SDM equivalents from isolated rainbow trout gut segments.

Gut segment	Bioavailability (%)[a]	Plasma concentration (μg/mL)[b]
Stomach/pyloric ceca (N = 3)	71.2 ± 8.6[c]	10.9 ± 4.2[c]
Proximal intestine (N = 3)	82.7 ± 7.4[c]	24.7 ± 5.6[c]
Distal intestine (N = 3)	93.0 ± 2.0[c]	34.6 ± 3.8[d]

[a]Over a 24-h interval.
[b]Plasma concentration at 24 h.
[c]Significantly different ($P \leq 0.05$).
[d]Significantly different from stomach but not proximal intestine ($P \leq 0.05$).

plasma concentrations rose from 20 to 80 μg/mL. These changes in bioavailability from 63 ± 9.2 to 50 ± 14.1%, however, were not found to be significant by the t-test. Bioavailability of SDM when measured by HPLC following administration of stable sodium SDM was 53 ± 16.1% (Fig. 3B). These results suggest that oral bioavailability in trout varied with SDM's chemical form (salt versus free drug) and was largely a result of the parent compound. Differences in bioavailability between the salt and free drug may be a direct result of a lower solubility of the free drug in water. Catfish exhibited a similar bioavailability pattern with the two chemical forms; however, the differences were not significant (Squibb et al. 1988).

Plasma levels of orally administered SDM in trout exceeded the 24-h minimum inhibitory concentration (MIC) (Bullock et al. 1974) for sensitive *Aeromonas salmonicida* strains for a duration of 10–75 h. The times noted were dependent upon the MIC values for individual bacterial strains. Plasma concentrations did not approach the 500 μg/mL levels indicated for SDM-resistant strains. Although MIC values are a benchmark of comparison, alterations imposed by in vivo binding and biotransformation make it difficult to equate plasma concentrations with antibacterial efficacy.

The distal intestine exhibited the greatest capacity to absorb SDM equivalents followed by the proximal intestine and the pyloric ceca region (Table 2). A 20% difference in bioavailability (disappearance from gastrointestinal tract), as demonstrated between the highest and lowest values, was reflected as a threefold difference in plasma [^{35}S]SDM levels. While the differences in plasma levels were significant, the physiological importance relative to the absorptive process of the whole gut is unknown, as the distal gut would be exposed only to residuals of the prior absorptive process. Although it is generally recognized that most drugs are primarily absorbed from the small intestine, these findings may gain significance if dietary formulation results in only partial release before reaching the distal intestine. The mechanism of these regional absorptive differences in the trout is unclear; however, it is known that in mammals, different circulatory routes exist for rectal regions as compared with the rest of the intestine (Schummer et al. 1981).

A low degree of plasma protein finding was found in both in vivo and in vitro binding studies. The percentage of plasma protein bound drug was consistent for 2, 6, 24, and 72 h following in vivo administration, with values of 16.6 ± 2.2, 16.2 ± 9.0, 13.5 ± 1.7, and 16.8 ± 1.0%, respectively. These findings suggest that changes in the metabolite profile and drug concentration during depuration were without influence upon protein binding. Furthermore, Scatchard analysis of a graded series of in vitro plasma/SDM incubations indicates that this binding was nonspecific in nature and nonsaturable through a concentration range of 0.2–10.0 mM. It is of interest to note that dose independency in binding has been demonstrated for a majority of the antimicrobial agents thus far examined in fish (Plakas et al. 1988; Squibb et al. 1988). However, the degree of protein binding has been shown to vary widely with different agents (Plakas et al. 1988; Squibb et al. 1988) and fish species (Guarino 1986). The low protein binding of SDM in the trout may facilitate elimination and contribute to the relatively large apparent volume of distribution as compared with mammalian species (Kleinow and Lech 1988).

Tissue distribution studies indicate that SDM was well distributed throughout the body tissues and fluids. SDM equivalents attained the highest levels in the bile followed by the intestine, liver, blood, skin, kidney, spleen, gill, muscle, and fat, respectively (Table 3). The $t_{1/2}\beta$ of [^{35}S]SDM in select tissues ranged from 5.0 to 13.9 d (Table 3). Peak SDM equivalent levels in organs and body fluids were attained by 12 h following oral administration for the blood, liver, bile, muscle, skin, spleen, gills, and kidney. Peak levels for the intestine and fat were delayed until approximately 18 h. A resurgence of fat [^{35}S]SDM concentrations well into the elimination phase provides for difficult interpretation. It can be noted that bile levels were maintained above 1000 μg/mL for up to 10 d post-administration. Data from in situ gastrointestinal preparations in the catfish suggest that SDM and particularly its N_4-acetylated metabolite undergo extensive enterohepatic recirculation (unpublished data). The protracted retention of SDM equivalents in the bile and the potential of recirculation suggest that consumable tissues may be exposed to additional drug significantly after administration. The significance of this process would be dependent upon the fish species, feeding status of the fish, biliary metabolites available, kinetics of the metabolites, and the drug concentration.

Results of mass balance studies indicate that clearance within the first 24 h by branchial and urinary routes of elimination accounts for less than 10% of the total dose administered (Table 4). These results in concert with the high biliary concentrations as evident in tissue distribution studies (Table 3) suggest that biliary excretion may play an important role in the elimination of SDM from fish.

Examination of metabolite profiles of the drug from mass balance studies and select tissues demonstrates a varying profile with route, biological matrix, and time (Tables 4 and 5). These studies demonstrate that for a comparable time (20 h) the majority of compound in the plasma is parent. Conversely under the same temporal conditions, N_4-A-SDM predominates in the bile, while the liver contains slightly greater amounts of N_4-A-SDM product than parent. These results support the view that the liver is instrumental in production and/or extraction of the N_4-A-SDM compound. A divergent metabolite profile is evident between branchial and urinary elimination products. The majority of branchial elimination occurred as parent whereas the urine contained a larger fraction as N_4-A-SDM.

These studies have demonstrated that SDM has a moderate-length $t_{1/2}\beta$ and is well distributed into extracellular water. SDM exhibits a wide tissue distribution and is concentrated into the bile as well as liver. Data for individual tissues indicate adequate clearance to meet mandated tolerances in accordance with recommended drug withholding times following single-dose administration; however, these studies indicate prolonged $t_{1/2}$'s

TABLE 3. Mean (SD in parentheses) tissue concentrations (μg/g or ppm) of total ^{35}S over time following a single oral administration of [^{35}S]SDM (42 mg/kg) to rainbow trout ($N = 4$). Values are presented with three significant figures except where magnitude or analytical detection limits require an alternative presentation. $t_{1/2}\beta$ (days) of terminal [^{35}S]SDM elimination phase in select organs: blood, 13.9; liver, 5.8; spleen, 9.9; kidney, 9.8; muscle, 11.9; gill, 12.7; skin, 5.0.

	Hours			Days							
Tissue	4	12	18	1	3	7	10	14	21	35	49
Blood	1.48 (1.21)	54.2 (20.00)	20.9 (5.48)	32.9 (3.57)	9.23 (1.18)	1.50 (0.44)	3.25 (0.73)	1.27 (0.63)	2.36 (0.86)	1.40 (0.24)	0.32 (0.11)
Gill	41.3 (37.70)	19.3 (2.58)	13.8 (2.77)	16.6 (4.11)	4.33 (0.62)	1.06 (0.34)	1.42 (0.57)	0.34 (0.13)	0.90 (0.19)	0.22 (0.12)	0.19 (0.22)
Liver	6.09 (6.48)	61.4 (3.49)	47.0 (9.17)	56.2 (12.39)	16.3 (7.08)	6.57 (3.61)	3.56 (1.52)	0.57 (0.21)	0.61 (0.30)	0.32 (0.12)	0.00 (0.00)
Muscle	0.73 (0.68)	13.3 (3.10)	10.4 (1.73)	11.2 (2.63)	3.98 (1.41)	0.78 (0.37)	1.28 (0.35)	0.02 (0.02)	0.25 (0.18)	0.42 (0.16)	0.06 (0.06)
Fat	0.37 (0.29)	7.60 (0.58)	21.7 (16.40)	10.8 (3.14)	3.63 (0.91)	3.25 (3.55)	2.46 (0.30)	0.63 (0.10)	4.93 (1.42)	3.80 (0.73)	0.24 (0.11)
Skin	1.04 (0.40)	30.6 (4.96)	32.9 (3.07)	31.9 (5.19)	8.04 (4.87)	1.74 (0.52)	1.42 (0.17)	0.10 (0.10)	0.48 (0.21)	0.44 (0.12)	0.08 (0.15)
Spleen	1.40 (0.73)	19.8 (7.47)	12.0 (3.58)	21.8 (4.15)	4.03 (0.88)	3.26 (2.87)	1.77 (0.16)	0.36 (0.36)	1.61 (1.15)	0.71 (0.23)	0.07 (0.13)
Kidney	1.16 (1.26)	24.7 (3.19)	20.8 (7.19)	26.9 (11.24)	3.76 (1.42)	1.04 (0.55)	1.75 (0.66)	0.39 (0.15)	0.28 (0.29)	0.09 (0.13)	0.11 (0.13)
Bile	40.7 (45.4)	1974.0 (246.6)	1750.5 (521.9)	2541.1 (1223.0)	1754.8 (238.1)	1924.5 (1405.6)	1112.8 (1495.5)	1.78 (0.30)	0.68 (0.31)	0.44 (0.16)	0.45 (0.32)
Intestine	10.1 (6.99)	71.6 (15.3)	123.1 (62.7)	122.0 (27.3)	34.1 (18.9)	31.0 (22.4)	37.7 (51.0)	0.12 (0.12)	0.77 (0.40)	0.48 (0.45)	0.07 (0.11)

TABLE 4. Relative contribution (means ± SD) of branchial and urinary routes to elimination of [^{35}S]SDM and N_4-A-SDM at variable times following i.v. administration of [^{35}S]SDM (42mg/kg) to rainbow trout ($N = 2$).

	Branchial		Urinary	
	Percent	Time (composite) (h)	Percent	Time (composite) (h)
Total drug eliminated	0.60 ± 0.24	25	1.98 ± 0.58	4
			5.46 ± 2.24	24
			7.80 ± 3.88	96
Chemical composition				
SDM	76.1 ± 13.0	25	32.9 ± 3.4	4
N_4-A-SDM	17.7 ± 6.0	25	52.2 ± 7.4	4

TABLE 5. Percent (means ± SD) of extracted ^{35}S radioactivity of SDM or N_4-A-SDM for bile ($N = 3$), plasma ($N = 2$), and liver ($N = 3$) 20 h following oral administration (42 mg/kg) of [^{35}S]SDM to rainbow trout. Totals based upon percent radioactivity recovered in SDM or N_4-A-SDM peaks.

Compound	Bile	Plasma	Liver
SDM	15.3 ± 4.2	84.2 ± 5.4	35.4 ± 9.0
N_4-A-SDM	85.8 ± 5.5	8.8 ± 4.5	55.3 ± 21.6

upon multiple dosing. Oral bioavailability of SDM was dependent upon chemical form and intestinal segment. SDM in trout exhibited low protein binding, significant metabolism, and differences in metabolic profile with tissue and route of elimination.

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LETTERS AND COMMENTS/ LETTRES ET COMMENTAIRES

PCB and Dioxin Research and Implications for Fisheries Research and Resource Management

The recently published experiments on the toxicity of 2,3,7,8-TCDD to lake trout (*Salvelinus namaycush*) have revealed the exquisite potency of this compound to this species (Walker et al. 1991). Eggs of the lake trout that contained 55 parts per trillion of 2,3,7,8-TCDD had increased egg and sac fry mortality making this among the most sensitive species of *all* vertebrates ever tested. In other studies, long-term monitoring of egg hatchability and viability of fry from female lake trout from various Great Lakes locations has shown that certain areas, particularly in Lake Michigan, are associated with a high incidence of embryo and fry mortality in certain years (Mac and Edsall 1991). Further research has shown that, like other vertebrates that are highly exposed to organochlorine contaminants in Great Lakes foodwebs, lake trout reproductive success is being affected by PCBs. The dioxin-like activity of PCB in four samples of Lake Michigan lake trout eggs ranged from at least 80 to 300 ppt expressed as toxicity equivalents relative to 2,3,7,8-TCDD (Smith et al. 1990). This finding should come as no surprise because 35 yr ago, researchers in upper New York State showed that the reproductive success of lake trout was severely affected by another organochlorine compound, DDT (Burdick et al. 1964). Isomer-specific chemical analysis has revealed the relationship between egg hatchability and dioxin-like components of the PCB. A recently published case history which discussed the evidence using an epidemiological approach concluded that maternally transferred PCBs reduce egg hatchability of Lake Michigan lake trout and that fry survival in lake trout is controlled by a severe mortality syndrome affecting fry just before swim-up and is reminiscent of the syndrome and associated behavioral signs observed 35 yr ago by the New York State researchers (Mac and Edsall 1991). These findings, together with reports of similar observations in other salmonines in other locations, were presented at a joint workshop held by the International Joint Commission and the Great Lakes Fishery Commission on September 24 and 25, 1990. Despite the weight-of-evidence, there was a reluctance among workshop participants to conclude that organochlorine compounds had caused the reproductive failure. Instead, the participants concluded that "maybe" organochlorine compounds were involved, reflecting a deep-seated reluctance of fisheries researchers to invoke toxic chemicals as a cause of injury to fisheries resources and a general pessimism about their ability to link causes with effects.

Fisheries research on toxic substances in the Great Lakes, and perhaps in North America generally, has been based on two premises: (1) that effects on fish populations are only likely to occur in very nearshore areas such as tributaries, harbors, and connecting channels and (2) that effects of toxic substances are thus likely to be much less important to inshore and offshore fish stocks than overfishing, physical habitat destruction, or the depredations by exotic species such as the sea lamprey (*Petromyzon marinus*) (Hartman 1988). This was based on an understanding of the locations of the toxic discharges, the entrapment of toxic substances on inshore sediments, and the less migratory behavior of nearshore fish species than inshore and offshore stocks. These two premises have led fisheries research on toxic substances into a classic "Catch 22" situation which has dominated funding for more than 20 yr and stifled progress. If a fisheries researcher believes the dual premise, he gets funded to do (1) laboratory research to develop water quality objectives to "prevent" damage to the fishery resource by "protecting the most sensitive species" or (2) surveys in rivers or harbors to determine, for instance, the incidence of liver tumors in brown bullhead (*Ictalurus nebulosus*) exposed to high levels of polynuclear aromatic hydrocarbons from steel-making operations. In this way, he does not have to look in the offshore areas to see whether anything has happened to the resource, and by not looking, he does not find any injury. If he does not accept the dual premise, he does not get funded and thus also does not get to look at the resource to see whether anything has happened and also does not find anything. Thus, these two premises have not only dominated fisheries toxic chemicals science but they have also remained unchallenged for a long period of time. As a corollary, persistent toxic substances have been regarded by fisheries managers as mainly a public health issue (Regier 1979), and health advisories and tolerances have been looked upon as an inconvenience caused by health authorities, interfering with the use of the resource.

These recent observations, publications, and workshops are beginning to challenge these premises and undermine the accepted body of knowledge on which these policies are still based. This challenge has immediate policy implications for research and resource managers. For instance, despite the implementation of an extensive sea lamprey control program costing tens of millions of dollars and the stocking with hundreds of millions of lake trout fingerlings, the Great Lakes Fishery Commission has been unable to achieve its stated objective of restoring self-sustaining populations of lake trout in the Great Lakes. It would seem that notwithstanding the availability of suitable spawning reefs, and controls on excess nutrients and on access to the resource, sexually mature lake trout are unable to produce viable young in most of the Great Lakes because of the levels of organochlorine contaminants in the eggs.

If it is accepted that certain organochlorine chemicals have affected lake trout reproduction, the question now posed by the new findings concerns when eggs and fry of salmonines and perhaps even all salmonids became nonviable in each of the Great Lakes. Analyses of radiodated sediment cores from several of the Great Lakes indicate that a variety of organochlorine compounds were entering the Great Lakes in substantial quantities at the same time that overfishing and sea lamprey were causing widespread depredations on lake trout, lake herring (*Coregonus artedii*), and lake whitefish (*Coregonus clupeaformis*) stocks in the 1930s, 1940s, and 1950s (Hartman 1988). Similarly, the extinction of the Lake Ontario deepwater sculpin

(*Myoxocephalus quadricornis*) between 1953 and 1964 occurred at the same time that loadings of 2,3,7,8-TCDD and other organochlorine compounds were exponentially increasing from U.S. chemical manufacturing along the banks of the Niagara River. Perhaps it is time to revisit some of the species extinctions that occurred on the Great Lakes during this century to reevaluate whether organochlorine compounds might be implicated as a significant component factor together with overexploitation, sea lampreys, and physical habitat destruction so that scientifically defensible policies can be developed for restoration and protection of Great Lakes fisheries resources.

Much of the funding and policies of the Canadian and U.S. governments, the Province of Ontario, and the eight Great Lakes states and of the Great Lakes Fishery Commission during the past quarter-century have been based on the dual premise outlined above. Perhaps it is also time for a radical reappraisal of funding and research objectives on toxic chemicals, since the original premises on which they are based are clearly no longer tenable. If it is accepted that actual effects are occurring that have injured the Great Lakes fisheries resources, research should be reoriented to investigate the actual causes of the injury using a coordinated epidemiological approach (Fox 1991). This forensic approach requires the expertise of a wide variety of disciplines that have not traditionally worked collaboratively, including population ecology, veterinary pathology, experimental toxicology, analytical chemistry and biochemistry, and sedimentology. To prepare a scientifically defensible case to link the injury to the cause is time consuming and resource intensive, but this is vital to the restoration of the valuable fisheries resources. In addition, novel ways must be found to reward researchers who have the courage to step off the treadmill of traditional aquatic toxicology, undertaken as controlled experiments in the laboratory, and to seek to answer the real toxicological riddles posed by the injured Great Lakes fisheries resources — Michael Gilbertson. *International Joint Commission, Great Lakes Regional Office, 100 Ouellette Avenue, Windsor, Ont. N9A 6T3, Canada.* (JB305)

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ANNOUNCEMENTS/AVIS

Canadian Conference for Fisheries Research (CCFFR)

Trent University
Peterborough, Ontario
January 3–4, 1993

Announcement of Conference and Call for Papers

This conference will be held in Peterborough, Ontario, January 3 and 4, 1993.

The major themes of the conference will be (1) Influence of fish and invertebrates on the ecological characteristics of their habitat; (2) Energy allocation patterns and strategies, ranging from the individual to the community level; and (3) Spatial and temporal distributions: methodology, patterns, and ecological implications.

A workshop is also planned on the following topic: Use of scientific information on fish for setting objectives towards fostering ecosystem integrity.

While papers on other themes may be submitted, authors are encouraged to adhere to the above themes as much as possible.

Deadline for abstracts is **August 30, 1992**. They should be typed (maximum 200 words, single spaced) and nature of presentation (oral/poster) indicated. A high quality of copy is required, suitable for photographic reproduction.

Abstracts should be addressed to Dr. Daniel Boisclair, Biological Sciences Department, University of Montreal, P.O. Box 6128, Station "A", Montreal, Que. H3C 3J7, Canada. Telephone: (514) 343-6762.

Conférence canadienne sur la recherche sur les pêches (CCRP)

Université Trent
Peterborough (Ontario)
les 3 et 4 janvier 1993

Avis de conférence et demande d'articles

La conférence aura lieu les 3 et 4 janvier 1993 à Peterborough (Ontario).

Les principaux thèmes abordés seront les suivants : 1) L'influence des poissons et des invertébrés sur les caractéristiques écologiques de leur habitat; 2) Les régimes et stratégies d'allocation de l'énergie, du niveau des individus jusqu'au niveau des communautés; et 3) Les distributions spatiales et temporelles : méthodologie, régimes et implications écologiques.

Il y aura aussi un atelier sur : L'application des données scientifiques sur les poissons à l'élaboration des objectifs pour promouvoir l'intégrité des écosystèmes.

Bien que des travaux sur d'autres thèmes puissent être présentés, nous incitons les chercheurs à s'en tenir autant que possible aux thèmes susmentionnés.

La date limite pour l'envoi des résumés a été fixée au **30 août 1992**. Les résumés doivent être dactylographiés (200 mots au maximum, à simple interligne). Il est important de préciser sous quelle forme se déroulera la présentation (présentation orale/affiche). Une copie de très bonne qualité est requise aux fins de la reproduction photographique.

Veuillez faire parvenir votre résumé à : D[r] Daniel Boisclair, Département des sciences biologiques, Université de Montréal, C.P. 6128, Succ. «A», Montréal (Québec) H3C 3J7, Canada. Téléphone : (514) 343-6762.

The Group for Aquatic Primary Productivity (GAP) 6th International Workshop

7–15 June 1993

National Hydrology Research Institute, Environment Canada, Saskatoon, Saskatchewan, Canada

Theme

Effects of physical forcing on primary production processes in inland and marine environments.

Based on a program of active experimentation and data analysis, the main objectives of the Workshop are to assess the states of knowledge on the Workshop theme, perform joint field experiments using different techniques to test their comparability and reliability, and define major gaps and urgent research needs.

Le Groupe de productivité primaire aquatique (GPA) 6[e] atelier international

7–15 juin 1993

Institut national de recherche en hydrologie, Environnement Canada, Saskatoon (Saskatchewan), Canada

Thème

Effets du forçage physique sur les processus de production primaire en milieux terrestre et marin.

S'inspirant d'un programme d'expérimentation active et d'analyse de données, les principaux objectifs de l'atelier sont évaluer les états des connaissances sur le thème de l'atelier, faire des expériences conjointes sur le terrain à l'aide de différentes techniques pour en vérifier la comparabilité et la fiabilité et définir les grandes lacunes et les besoins urgents de recherche.

Keynote Presentations:

Physics

The physical environment and its affects on primary production processes. E. C. Carmack, Institute of Ocean Sciences.

Chemistry

Nutrient dynamics and primary production across frontal systems. P. J. Harrison, University of British Columbia.

Biology

Biological responses to physical forcing, with emphasis on photoinhibition and light/shade adaptation. P. J. Neale, University of California, Berkeley.

Modelling

Modelling the interaction between physical and biological processes. J. J. Cullen, Dalhousie University.

Experimental Program

Group I: Physics; experimental leaders: B. Kenney and E. C. Carmack. Group II: Nutrient stimulation; P. A. Chambers and J. G. Stockner. Group III: Light/shade adaptation; R. D. Robarts and P. J. Neale. Group IV: P/R quotients; M. L. Bothwell and T. Bott.

A fifth group on Modelling could be set up if there is enough interest.

Experimental Sites

Redberry Lake (110 km northwest NW of Saskatoon, TDS ~14 $g \cdot L^{-1}$, typical June primary production ~30 mg $C \cdot m^{-1} \cdot h^{-1}$); South Saskatchewan River (oligotrophic above Saskatoon, N and P gradients downstream for ~50 km); laboratory (well-equipped modern laboratory at National Hydrology Research Institute); if sufficient interest is expressed, shallow, hypertrophic Humboldt Lake (120 km northeast of Saskatoon) may be visited.

General Information

Participation in GAP 6 will be limited to 80 persons. Contributed papers will be limited to poster presentations (maximum 100 × 200 cm). Accommodation will be on the University of Saskatchewan campus (~$27/night). Workshop fees will be about $200.

The 1993 ASLO meeting will be held in Edmonton, Alberta, from 30 May to 3 June. Following the post-ASLO field trips, a bus will take GAP participants to Saskatoon (5 hours) on 6 June for a nominal fee if there is enough interest.

Persons wishing to participate in GAP 6 are asked to contact the Chairman before 1 June 1992: Richards Robarts, GAP 6 Chair, National Hydrology Research Institute, Environment Canada, 11 Innovation Blvd., Saskatoon, Sask. S7N 3H5, Canada. Telephone: (306) 975-6047; Fax: (306) 975-5143.

Présentation clés

Physique

Le milieu physique et ses effets sur les processus de production primaire. E. C. Carmack, Institut des sciences de la mer.

Chimie

Dynamique des matières nutritives et production primaire dans les systèmes frontaux. P. J. Harrison, Université de la Colombie-Britannique.

Biologie

Réponses biologiques au forçage physique, avec accent sur la photoinhibition et l'adaptation à la lumière et à l'ombre. P. J. Neale, Université de la Californie, Berkeley.

Modélisation

Modélisation de l'interaction entre les processus physiques et biologiques. J. J. Cullen, Université Dalhousie.

Programme expérimental

Groupe I : Physique; directeurs d'expérience : B. Kennedy et E. C. Carmack. Groupe II : Stimulation de nutriments; P. A. Chambers et J. G. Stocker. Groupe III : Adaptation à la lumière/ombre; R. D. Robarts et P. J. Neale. Groupe IV : Quotients P/R; M. L. Bothwell et T. Bott.

Un cinquième groupe de modélisation pourrait être créé sur demande.

Sites expérimentaux

Lac Redberry (110 km au nord-ouest de Saskatoon, MTD ~ 14 $g \cdot L^{-1}$, production primaire type de juin ~ 30 mg $C \cdot m^{-1} \cdot h^{-1}$); rivière Saskatchewan Nord (oligotrophe en amont de Saskatoon, gradients de N et P en aval sur ~ 50 km); laboratoire moderne et bien équipé à l'Institut national de recherche en hydrologie; visite possible, si cela suscite assez d'intérêt, du lac Humbolt (lac hypertrophe peu profond à 120 km au nord-est de Saskatoon).

Information générale

La participation au GPA 6 sera limité à 80 personnes. Les communications seront limitées à des affiches (100 × 200 cm maximum). L'hébergement se fera sur le campus de l'Université de la Saskatchewan (~ 27 $/nuit). Les frais d'inscription aux ateliers seront d'environ 200 $.

La réunion de 1993 de l'ASLO aura lieu à Edmonton, en Alberta, du 30 mai au 3 juin. Après les excursions qui suivront l'ASLO, un autobus transportera les participants du GPA à Saskatoon (5 heures) le 6 juin à un prix modique si cela suscite assez d'intérêt.

Les personnes désireuses de participer au GPA 6 sont priées de communiquer avec le Président avant le 1er juin 1992 : Richard Robarts, président du GPA 6, Institut national de recherche en hydrologie, 11, boul. Innovation, Saskatoon (Saskatchewan) S7N 3H5, Canada. Téléphone : (306) 975-6047; télécopieur : (306) 975-5143.

19th Annual Aquatic Toxicity Workshop

The 19th Annual Aquatic Toxicity Workshop will be held at the Edmonton Hilton in Edmonton, Alberta, October 4–7, 1992. The themes for the Workshop are anthropogenic discharges into northern aquatic environments; new toxicity testing methods: approaches and evaluation; and pathways and fate

19e atelier annuel de la toxicité aquatique

Le 19e atelier annuel de la toxicité aquatique aura lieu au Edmonton Hilton à Edmonton en Alberta du 4 au 7 octobre 1992. Les thèmes de l'atelier sont déversements anthropogéniques dans l'environnement aquatique du nord; nouvelles méthodes d'épreuves de toxicité : méthodes et évaluation; et le

of contaminants in the aquatic environment. Major topics will include: (1) QA/QC; (ii) risk; (iii) multitier testing; (iv) groundwater contamination; (v) fate processes; (vi) pulp and paper industry; (vii) petroleum industry; (viii) hazardous and special wastes; (ix) long-range transport; and (x) ecosystem evaluation. A plenary session will be held on the themes of the Workshop on the morning of October 5.

Platform and poster sessions will be accepted in English or French, and abstracts must be received by **July 1, 1992**. For further information, please contact Earle G. Baddaloo, Program Chair, Standards Research and Development Branch, Environmental Assessment Division, Alberta Environment, 6th Floor, 9820-106 Street, Edmonton, Alta. T5K 2J6, Canada. Telephone: (403) 427-6102; facsimile: (403) 422-9714.

parcours et le devenir des contaminants dans l'environnement aquatique. Les sujets principaux incluront i) assurance de la qualité/contrôle de la qualité; ii) le risque; iii) épreuves à multiples niveaux; iv) la contamination de l'eau souterraine; v) les procédés du devenir; vi) l'industrie de pulpe et papier; vii) l'industrie pétrolière; viii) déchets hasardeux et spéciaux; ix) cheminement des déchets à longue portée; x) l'évaluation de l'écosystème; et xi) applications statistiques en toxicologie. Une session plénière des thèmes de l'atelier aura lieu le matin du 5 octobre.

Les séances d'affichage et d'exposés seront acceptés en anglais ou en français et les résumés soumis au plus tard **le 1 juillet 1992**. Pour de plus amples renseignements communiquer avec Earle G. Baddaloo, Président du comité du programme, direction de recherche et développement, section de l'évaluation de l'environnement, Environnement Alberta, 6^{e} étage, 9820, 106^{e} rue, Edmonton (Alberta) T5K 2J6, Canada. Téléphone : (403) 427-6102; télécopieur : (403) 422-9714.

5th International Conference on the Conservation and Management of Lakes

17–21 May 1993, Stresa, Italy

Strategies for Lake Ecosystems beyond 2000

The Conference is organized by Istituto Italiano di Idrobiologia (CNR), Istituto di Ricerca Sulle Acque (CNR), the International Lake Environment Committee Foundation (ILEC), and the International Association Water Pollution Research Control (IAWPRC).

The aim of the Conference is to focus on some of the major problems related to the protection and use of lake ecosystems considered as a primary resource for economic development. The programme will include invited lectures and offered papers and posters, which will be published in the Conference proceedings.

Three main topics will be addressed:

- Updating of the scientific basis of lake functioning in natural, stressed, and recovered situations as a management tool (scientific basis for managing eutrophication; acid rain and the effects on aquatic ecosystems on a global scale; water quality in lakes and reservoirs for human use; fate and effects of in-lake micropollutants; non-point-source control for nutrients)
- Political and administrative aspects of the management of lakes as a primary resource (scientific findings and their utilization at the socioeconomic and administrative levels for lake/reservoir management)
- Role of environmental education in involving citizen organizations to support the protection of lakes (environmental education; citizen participation)

The venue will be the Palazzo di Congressi, Stresa, Lago Maggiore. Stresa has good railway connections with three international airports: Milano-Linate (90 minutes), Milano-Malpensa (60 minutes), and Genève (150 minutes).

The official language of the Conference will be English.

5th International Conference on the Conservation and Management of Lakes

du 17 au 21 mai 1993 à Stresa, Italie

Strategies for Lake Ecosystems beyond 2000

La conférence est organisée par l'*Istituto Italiano di Idrobiologia (CNR)*, l'*Istituto di Ricerca Sulle Acque (CNR)*, l'*International Lake Environment Committee Foundation (ILEC)* et l'*International Association Water Pollution Research Control (IAWPRC)*.

Cette conférence a pour objet de mettre l'accent sur certains des plus importants problèmes liés à la protection et à l'utilisation des écosystèmes lacustres en tant que ressources primaires de développement économique. Le programme comprendra des conférences données par des conférenciers invités de même que la présentation d'articles et d'affiches qui seront publiés dans le compte rendu de la conférence.

Trois grands sujets seront traités :

- La mise à jour des connaissances scientifiques fondamentales sur les lacs en situations naturelles, de stress ou de rétablissement en tant qu'outil de gestion (fondements scientifiques de la gestion de l'eutrophisation; pluies acides et leurs effets sur les écosystèmes aquatiques à l'échelle du globe; qualité de l'eau des lacs et des réservoirs destinée à être utilisée par l'homme; devenir et effets des micropolluants présents dans les lacs et limitation non ponctuelle des matières nutritives)
- Aspects politiques et administratifs de la gestion des lacs en tant que ressources primaires (données scientifiques et leur utilisation socio-économique et administrative en gestion des lacs et des réservoirs)
- Rôle de l'éducation en matière d'environnement dans le contexte de l'appui accordé par les organisations de citoyens à la protection des lacs (éducation écologique; participation de la population)
- La conférence se tiendra au Palazzo di Congressi, Stresa, Lago Maggiore. La ville de Stresa est facilement accessible par train à partir des aéroports internationaux de Milano-Linate (90 minutes), de Milano-Malpensa (60 minutes) et de Genève (150 minutes).

L'anglais sera la langue officielle de la conférence.

For further information, contact the Secretariat, R. M. Società di Congressi s.r.l., Via Ciro Menotti 11, 20129 Milano, Italy. Telephone: 02/70126308-70126772; facsimile: 02/7382610.

Pour de plus amples renseignements, veuillez entrer en relation avec le secrétariat de la conférence à l'adresse suivante : R. M. Società di Congressi s.r.l., Via Ciro Menotti 11, 20129 Milano, Italie. Téléphone : 02/70126308-70126772; télécopieur : 02/7382610.

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Editor-in-Chief:

H.J. Dumont, *Ghent, Belgium*

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Journal Highlight

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Canadian Journal of Fisheries and Aquatic Sciences

Journal canadien des sciences halieutiques et aquatiques

Volume 49, No. 5, May 1992

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